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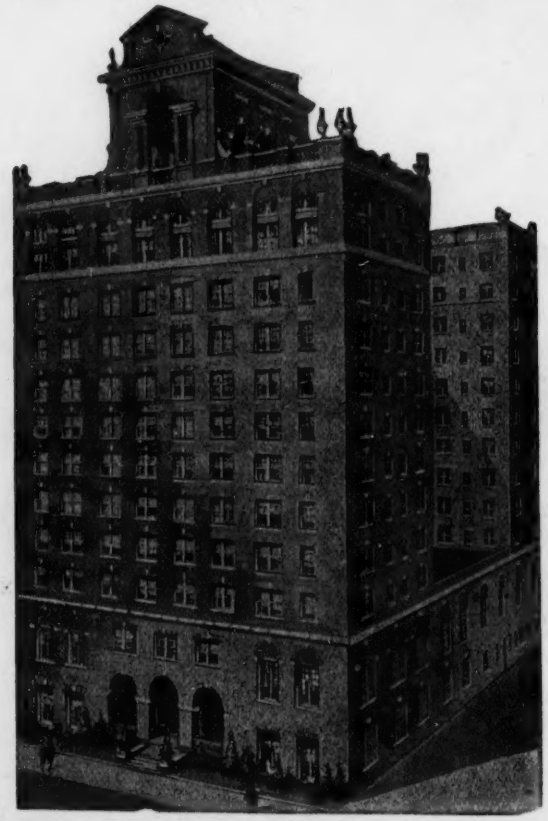

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PHYTOPATHOLOGY

VOLUME 22 OCTOBER, 1932 NUMBER 10

A STUDY OF TWO SEPTORIA LEAF SPOTS OF CELERY[1]

L. C. COCHRAN

(Accepted for publication February 29, 1932.)

INTRODUCTION

Late blight is one of the most serious diseases of celery in the United States. During a preliminary study of the disease in the fall of 1928, it was pointed out to the writer by Ray Nelson of the Michigan Agricultural Experiment Station that he had had under observation for 8 to 10 years 2 distinctly different types of spots caused by late blight on celery. Upon further examination, it was found that there are 2 distinct forms of the disease caused by 2 distinct species of the genus Septoria. It is the purpose of this paper to present studies that will help to distinguish these fungi and to clarify nomenclature and life histories.

HISTORY OF LATE BLIGHT

According to Chupp (7, p. 89), late blight of celery is probably about as old as the cultivation of celery and may have been observed as early as 1840. The first published report of it as a distinct disease was not made until 1890, when it was discovered in Italy by Briosi and Cavara. The disease was first recognized in the United States by Chester (4)(5) in Delaware in 1891. Rostrup (16) identified the disease in Denmark in 1893. Prillieux and Delacroix (22) found the disease in France in 1894, and Sorauer (26) in Germany in 1896. Pethybridge (21) and Chittenden (6) reported the disease as serious in all parts of the British Isles in 1910.

Shortly after Chester found Septoria on celery in Delaware, it was reported from almost all the important celery-growing regions in the United States. Duggar and Bailey (12) reported it as the cause of enormous losses in central and western New York in 1897. Rogers (23) accredited the disease with serious losses in California in 1897. Extreme losses occurred in California-shipped celery during the winter of 1908, when 1,950 cars, valued

[1] Journal article 84 (n.s.) from the Agricultural Experiment Station of Michigan State College, published by permission of the Director.

This paper includes material presented by the writer as a thesis in partial fulfillment of the requirements for the M.S. degree, Department of Botany, Michigan State College, June, 1930.

The writer wishes to express his indebtedness to Dr. E. A. Bessey, under whose direction this work was done, for correcting the manuscript and for invaluable advice and suggestions. He also is indebted to Dr. Ray Nelson for many suggestions throughout the work and for help with the photographic work.

at $550,000 were lost. Coons and Levin (9) and Coons (8) reported a loss estimated at over $1,000,000 for Michigan alone in 1915. In a survey of market diseases, Link and Gardner (19) found heavy losses in shipped celery and celeriac in 1918.

Since the first published note upon late blight, its occurrence has been reported until at the present time Chupp (7) and Owens (20), in their textbooks, state that it is the most cosmopolitan of all celery diseases. Thus in 40 years, the disease has become the most serious malady of celery.

A study of the literature showed that Dorogin (11), in 1915, had observed two organisms causing two distinct types of the disease in Russia. He identified one with the typical form reported from other regions, but considered the other a new species and gave it a new name. Foster and Weber (14) described two sizes of leaf spots caused by Septoria on celery in Florida in 1924, but they agreed with Laibach (18) and regarded these as strains of the organism described by Briosi and Cavara and not as separate species.

Aside from these reports and the observations of Nelson, mentioned above, only one species of Septoria is usually listed as a parasite on celery. (Anderson (2), Seymour (25)). Since the writer wishes to present studies that will differentiate the two forms before discussing the question of names, temporary names or symptom names will be used to designate the forms in this paper. One of the fungi produces a larger leaf lesion than the other and will be designated as the large-spot form and the other the small-spot form.

SYMPTOMS

Under optimum conditions, a period of from 9 to 12 days is necessary for these fungi to enter the host and establish themselves to the extent of the production of visible lesions. Under adverse conditions of temperature, moisture, or health of the host, this period may be lengthened but is never shortened. Early symptoms of infection caused by the 2 fungi are very similar, but, as they develop, the lesions soon become easily distinguishable.

The large-spot form (Fig. 1, B and Fig. 2, A) usually requires an incubation period of a day or 2 less than that of the small-spot form. The first visible evidence of infection is in the form of a small chlorotic fleck. The first definite lesion is marked by collapse of the host tissue containing and adjacent to the fungus. It seems that the fungus may at a given state in its development secrete some toxic substance that actually kills the host cells for some distance, and this is followed by collapse of tissue, even when the hyphae are not present. This type of lesion in its early stages is very similar in appearance to that caused by *Cercospora apiicola*. In general, the spots are from 3 to 6 mm. in diameter. If the atmosphere is very moist, small, black, scattered pycnidia may become evident in the center of the spot 2 or 3 days after the first signs of collapse. These pycnidia are never

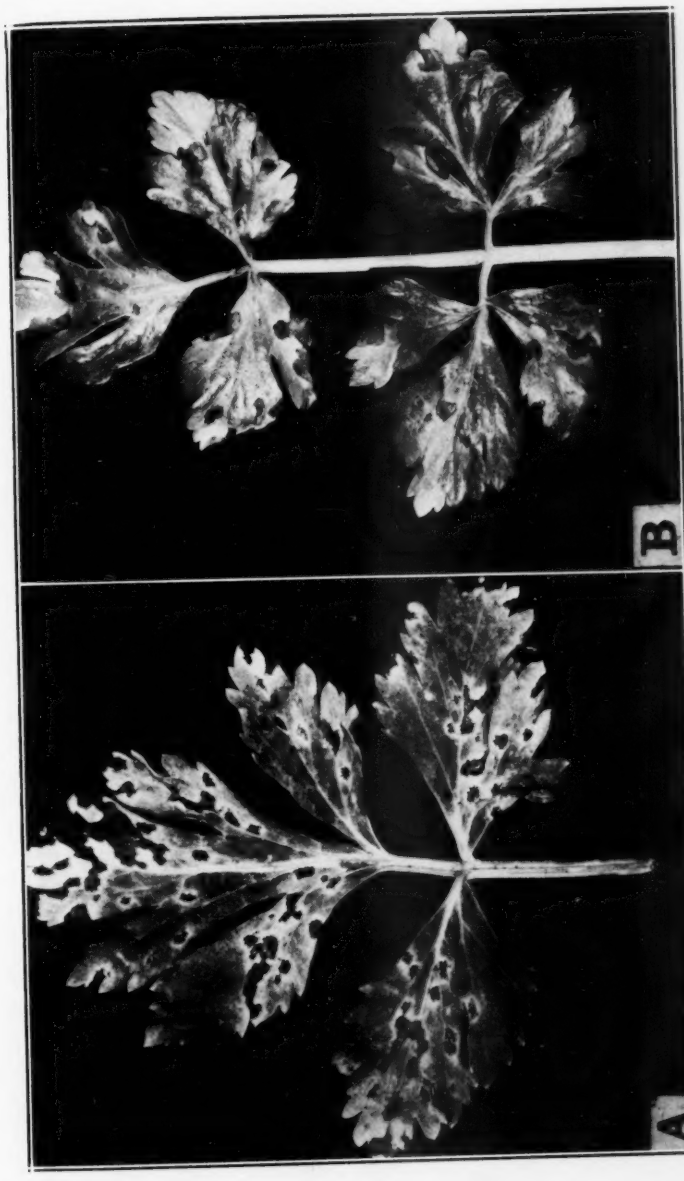

FIG. 1. A. Celery leaf infected with small-spot form of Septoria (*S. apii graveolentis*). ×2. B. Celery leaf infected with large-spot form of Septoria (*S. apii*). ×3.

FIG. 2. A. Celery leaflet, showing stages in the development of the lesions of *S. apii*. ×4. The spot near the point of the arrow shows scattered pycnidia in the center. B. Celery leaflet, showing stages in the development of the lesions of *S. apii graveolentis*. ×4. Some of the spots show pycnidia in uncollapsed tissue. In all cases the pycnidia are clustered and many are actually fused.

found along the margin of the spot and are scattered singly, never clustered. If the weather continues cool and moist, the spot may increase to 2 or 3 times its original size. If but a single lesion is present on a leaf, the leaf may not show much change for a week, but 2 or 3 lesions are sufficient to cause yellowing in as many days.

The small-spot type (Fig. 1, A and Fig. 2, B) differs from the large in that as soon as, or sometimes even before, chlorotic spots appear on the leaves fruiting bodies may be found. Pycnidia may be found outside of the indefinite margin of the spot in apparently healthy tissue. The killing and collapse of the tissue start at the middle of the spot and progress outward. The pycnidia that were buried in the leaf parenchyma are now exposed as erumpent black bodies closely crowded together and often fused together in twos and threes. In general, the spot is from less than ½ mm. to 2 mm. in diameter and never attains the size of the large-spot type. The easy-blanching varieties of celery are able to endure from 3 to 5 of these spots. The green celeries seem to be able to tolerate numerous spots before yellowing occurs. If the infection is severe in damp weather, the spots may fuse, giving a brownish black appearance, and the leaf rots with a soft, wet root without yellowing.

The striking differences between the 2 types of spots are, thus, the size and the time and manner of production of fruiting bodies. The mature or completely developed spot of the large-spot type is definite in outline, brown to reddish brown in color, and surrounded by a darker reddish brown border, with scattered pycnidia near the center. The small-type spot is indefinite in outline, brownish to black or sometimes gray, with black pycnidia that may be found both in the necrotic portion of the spot and often in the green tissue surrounding it. The pycnidia in the spot are erumpent, closely crowded, and some are fused in twos and threes. Although the difference in size of the spots of the 2 types is quite striking, a comparison can only be relative, since spots produced by either of the fungi may increase in size for several weeks or even until the host is entirely killed. Comparing the 2 under similar conditions, the lesion produced by the small-spot form on leaves ranges from ½ to 3½ mm. in diameter, and the large type may attain a diameter from 1½ to 10 mm. In the field, the small-spot type is usually ⅓ to ½ the diameter of the large one.

ETIOLOGY

Both Septorias were collected on celery from the Kalamazoo marshes, and isolations from these were used in cultural wark. Single-spore cultures were made by the dilution-plate method. After 2 weeks of incubation, spore suspensions of each form were made, and 5 healthy plants in separate infection chambers were atomized with each. The plants were allowed to remain in the propagating cases for 48 hours, when they were removed to the green-

house bench. After 13 days both lots of plants showed typical types of the disease, while the checks remained entirely healthy. Isolations from lesions gave characteristic growth on potato-dextrose agar for both forms of the fungus. In the course of this study pure-culture inoculations were repeated many times.

To avoid individual differences in celery plants, which might be argued as a cause of the difference in size of spot caused by the two fungi, the two halves of a single plant were atomized with spore suspensions of the two forms, respectively. After two weeks the characteristic types of spots were apparent on their respective sides.

MORPHOLOGY OF THE TWO SPECIES OF SEPTORIA AND THEIR RELATION TO HOST TISSUE

The mycelia of these two forms of Septoria in sections of diseased leaves are very similar and are composed of twisted and interwoven, irregularly septate, brownish to black, irregularly thickened hyphae. At the corners of the host cells, where the mycelium is intercellular, there may be whole fascicles of hyphae producing knot and bulb-like formations of irregular outline. The mycelium of the small-spot form is from 1.5 to 4.5 μ in diameter, while that of the large-spot form is 1.0 to 5.5 μ in diameter.

In sections through lesions the mycelium of the small-spot form is found to be much more extensive than that of the large one. Strands of mycelium may be found as far as 1,500 μ beyond the edge of the collapsed tissue. Absence of the mycelium in portions of the lesions of large-spot type points to the possible production of a toxic substance that kills the tissues in advance of the fungus and helps to explain the shorter incubation period and the increased size of the spot produced by the large-spot form.

The pycnidia of the 2 forms differ in size and shape and in their time and place of appearance. The pycnidia of the small-spot form are visible very early and are one of the first signs that distinguish a lesion, while the pycnidia of the large-spot form are seldom evident on well-aerated leaves and, when present, occur only in the central portion of the lesion. Pycnidia of the small-spot form range from 73 to 147 μ[2] in diameter, with ostioles of from ⅓ to ½ the diameter of the pycnidia. The pycnidia of the large-spot form are smaller, ranging from 65 to 95 μ in diameter, with ostioles of less than ¼ the diameter of the pycnidia. The pycnidia of the large-spot form are practically spherical and are always borne singly unless in very old lesions that have been in a moist atmosphere, but those of the small-spot form are usually crowded and even fused.

The spores (Fig. 3) or conidia of both types are long, slender, filiform, hyaline, faintly septate bodies. The spores of the 2 fungi differ markedly

[2] The measurements were made on pycnidia on leaves that had been cleared in carbol-turpentine.

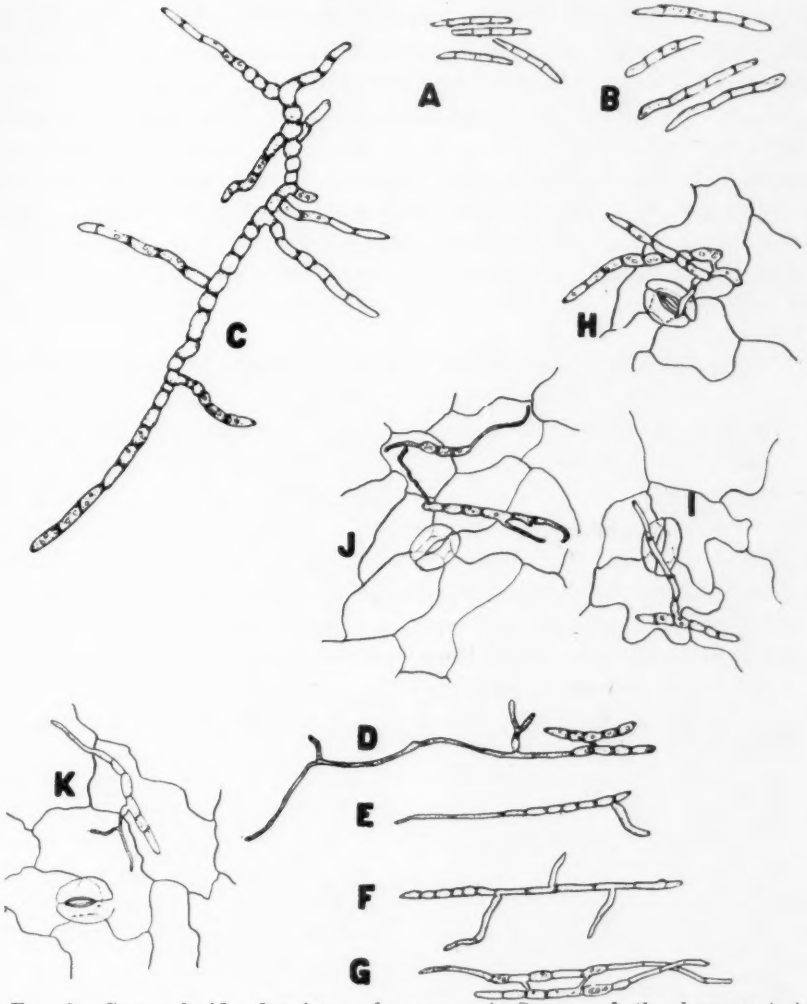

FIG. 3. Camera-lucida drawings of spores. A. Spores of the large-spot type Septoria (*Septoria apii*). B. Spores of the small-spot type Septoria (*S. apii graveolentis*). C. 3-day-old germinating spore of the small-spot type. D. and G. Germinating spores that have fused. E. and F. Germinating spores of large-spot type. H. Germinating spores with germ tube passing over a stoma. I. Germ tube passing directly over a stoma J. Fused spores on epidermis. K. Germinating spore with germ tube entering a cell through the entielmal wall.

in size, shape, and appearance. The spores of the small-spot form (Fig. 3, B) are slightly flexuose, blunt at the ends, granulose, with several indistinct septa, and vary from 22.5 to 58.5 μ in length by 1.5 to 3.0 μ in diameter. The mean for length was 35.0 μ and 2.2 μ for diameter. In plotting a curve of the measurements of the spores, it was found that 92 per cent would be included in lengths between 28.5 and 50.2 μ and 66 per cent between 31.5

and 42.7 μ. The number of septa varied from 0 to 7. Ten per cent had less than 3 septa, 60 per cent had 3 septa, 18 per cent 4 septa, 10 per cent 5 septa, and 2 per cent had more than 5 septa.

The spores (Fig. 3, A) of the large-spot form were straight or slightly curved, minutely granular, with indistinct septa. In size the spores varied from 13.5 to 34.2 μ in length and from 1 to 2½ μ in diameter. The mean was 24.6 μ for length and 1.8 μ for diameter. In plotting a curve as before, it was found that 86 per cent of the spore lengths were between 21 and 28.5 μ. The number of septa varied from 0 to 4. One and one-half per cent had less than 2 septa, 34 per cent had 2 septa, 64 per cent had 3 septa, and ½ of 1 per cent had 4 septa.

The above measurements were made from a fresh spore suspension taken from spots of several plants and prepared in the following way: Small sections of diseased spots on leaves from isolated plants that had been infected with pure cultures of the organism were placed in a test-tube containing sterile water. The tube was agitated so that the mature spores were washed from the cirri on the ostioles of the pycnidia. The suspension was placed on a slide and the spores were measured under oil immersion. Septa of fresh spores were very indistinct, so the septation counts were made on spores that had been fixed and stained with gentian violet. One thousand spores of each type were measured.

SEARCH FOR PERFECT STAGE

Stevens (27) and Gäumann (15) report several Septoria species other than those on celery, which in their perfect stage are members of the genera Leptosphaeria and Mycosphaerella. Klebahn (16) found a Pleospora on overwintered celery leaves but failed to get infection when he sprayed the ascospores on healthy celery leaves. The writer observed perithecia of Leptosphaeria and Ophiobolus in old Septoria lesions on overwintered celery leaf stalks, but single ascospore cultures on potato agar did not produce growth characteristic of either Septoria species.

Stevens (27) reported success with ultra-violet light in inducing the perfect stage of certain Ascomycetes. The writer exposed single-spore cultures and single-spore-mated cultures of the two types of Septoria in open Petri dishes at 50 cm. from a mercury vapor lamp, but no evidence of an ascigerous stage was observed. At exposures above 20 seconds the fungi were either killed or inactivated so that their growth was greatly reduced.

Cultures of both of the fungi on standard laboratory agars along with cultures on steamed celery stems and leaves were stored at 2–3° C. for a period of 16 weeks. The cultures were examined from time to time, but a perfect stage was not found, although there appeared sclerotium-like mats in the bottom of the tubes of the steamed celery-leaf and leaf-stalk cultures of both types.

CULTURAL CHARACTERISTICS

In pure culture both fungi were found to grow well on many of the common laboratory media. The following media were used: potato-dextrose agar, prune agar, Coons' synthetic agar, hard oat agar, corn-meal agar, nutrient dextrose agar, celery-leaf-stalk-decoction agar, horse-dung-decoction agar, steamed celery-leaf stalks, carrot plugs, sterile filter paper, steamed water-cress stems, steamed parsley leaves, and steamed muck. The amount of growth on the various media varied in proportion to the amount of nutrient afforded the fungi, but the type of growth produced by the two fungi was quite markedly different (Fig. 4). The large-spot form produced

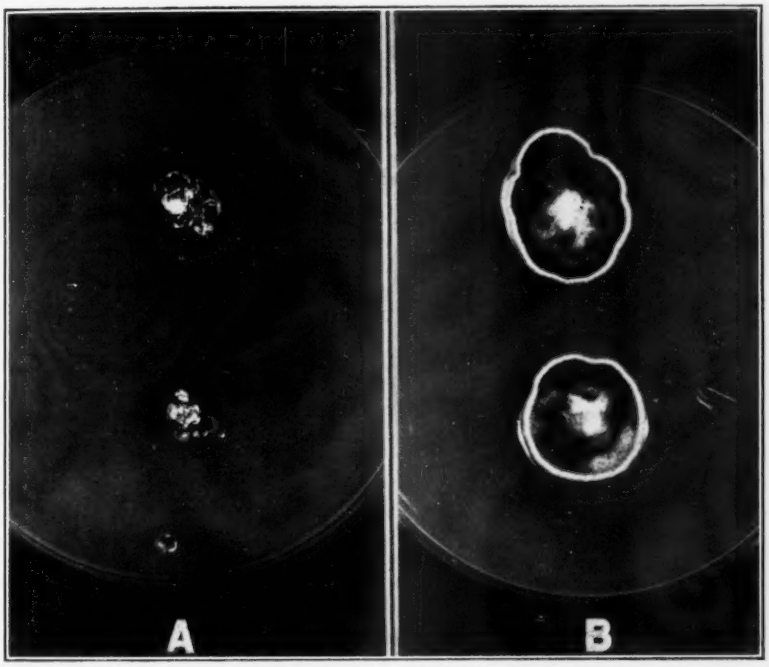

FIG. 4. Fifteen-day-old cultures on potato-dextrose agar. A. *Septoria apii graveolentis*. B. *S. apii*.

a colony of nearly twice the size of that of the small-spot form under the same conditions, and the growth was of a more spreading type, with fewer fruiting bodies. The small-spot form produced a more tufted growth of somewhat cheesy consistency that, upon examination, proved to be masses of pycnidia. Both fungi produced brownish black submerged mycelia and white aerial mycelia. The submerged mycelium of the small-spot form was quite ropy as compared to that of the large form. On potato-dextrose agar the large-spot organism produced a purple region in the medium around the colony, but the small-spot form produced no color.

Neither of the fungi was able to grow on sterile muck, and only a very scant growth occurred on corn-meal agar. The large-spot form grew luxuriantly on all the other media except the celery-decoction agar, where the growth was very scant and no fruiting bodies were formed. On this same agar the small-spot form grew and produced considerable mycelium with clumps of pycnidia. In a later experiment it was noticed that the large-spot form did not infect celery-leaf stalks. Since the base infusion of the celery-decoction agar was made by steaming celery-leaf stalks in water, it would seem that the growth of the fungus on agar might be correlated with that on the celery plant. However, both fungi grew equally well when they were placed on whole celery-leaf stalks that had been steamed.

THERMAL DEATH POINTS OF SPORES

As a further point of distinction it was found that the spores of the 2 fungi differed in their ability to withstand heat. Suspensions of mature spores in capillary tubes were exposed to a range of temperatures in water baths for a period of 10 minutes and then plated in potato agar. The results are recorded in table 1.

TABLE 1.—*Thermal death point of spores, 10 minutes in capillary tubes*

Temperature	40° C.	41° C.	42° C.	43° C.	44° C.	45 °C.	Check
Small-spot form	+++[a]	+	–	–	–	–	+++
Large-spot form	+++[a]	++	+++	++	–	–	+++

[a] +++ indicates numerous colonies developing, + indicates only a few, and – indicates no growth at all.

VEGETATIVE GROWTH AT VARIOUS TEMPERATURES

The season in which the diseases appear in the field, together with their reactions to controlled conditions in the greenhouse, suggests a rather sharp susceptibility on the part of the parasites to high temperatures. Thomas and Muller (29) and Campanile (10) found better growth of the celery Septoria at from 13° to 19° C. than at from 22° to 25° C. Owens (20) says that the disease caused by Septoria on celery is a cool-weather disease, the fungus thriving best between 60° and 70° F. To obtain further information on the temperature relations of the fungi, use was made of a modification of a Ganong differential thermostat. This piece of apparatus consists of low- and high-temperature chambers, between which are 12 small compartments that range in temperature from that of the low-temperature chamber to that of the high one.

Plantings of both types of the celery Septorias were placed on respective halves of Petri dishes on potato-dextrose agar and 3 dishes placed in each comprtment. The average temperature and growth after 18 days are recorded in table 2.

The 2 fungi are shown in table 2 to be distinctly different in their temperature requirement for growth. The large-spot type grows at tem-

TABLE 2.—*Growth of celery Septorias (on potato-dextrose agar) after 18 days at various temperatures*

Compartment number	Temperature range ° C.	Large-spot form		Small-spot form	
		Av. diam. of colony in mm.	Character of growth	Av. diam. of colony in mm.	Character of growth
1	9–12	7.0	very slight growth	none	tufted
2	11½–15	7.5	"	very slight	small white
3	17–19	18.0	good growth	6.5	aerial mycelium good growth
4	20–21	21.0	"	6.0	"
5	22–23	26.0	luxuriant growth	6.5	"
6	23–24	26.0	very black	5.0	"
7	24–25	23.0	no aerial growth	slight	small and black
8	26–27	13.0		none	
9	27–28	only a few filaments		"	
10	28½–30	none		"	
11	31–34	"		"	
12	38–42	"		"	

peratures between 10° and 27° C. with an optimum of between 22° to 24° C. The small type grows at temperatures between 14° and 25° C. with an optimum between 18° to 22° C. The inhibition of growth at low temperatures points to the possibility of control of the small-spot type, which causes serious damage to celery in storage, by lowering the temperature of the storage chamber to a point at which the fungus will not grow.

EFFECT OF THE REACTION OF THE MEDIUM

Both of the celery Septorias grew well on a wide variety of media, which also varied widely in hydrogen-ion concentration. To test the reaction of the fungi to differences in pH, a series of media was prepared in the following manner. Since it was impossible to harden agar in solutions of high acidity, a liquid medium was used. The ordinary base of Coons' synthetic medium was used and sterile N/10 NaOH or HCl was added to make a variable series as determined by the colorimetric method. The reaction of the medium varied from pH 3.0 to 8.8 by 0.2 point intervals. Thirty cubic centimeters of the base solution were placed in 75 cc. Erlen-

meyer flasks in which small filter-paper cones had been placed, and the flasks were autoclaved. The culture flasks were inoculated by placing on the tip of the cone a small piece of agar containing mycelium. Both fungi grew well throughout the series. The large-spot type grew slightly more luxuriantly on the acid side of the scale, but the small-spot type showed no specificity. Campanile (10), working with what probably is the large-spot fungus of this study, reported more spread of the fungus on neutral or slightly alkaline media.

SPORE GERMINATION AT DIFFERENT TEMPERATURES

A suspension of mature spores in tap water was obtained from leaves and placed in hanging drops on the under sides of glass slides that had been rimmed with vaseline. The slides were placed on supports in Petri dishes that had been lined with moist filter paper and the dishes were placed in the compartments of the Ganong thermostat. The slides were allowed to remain exposed to the different temperatures for a period of 14 hours, when they were removed and the germination stopped in the following manner. The slides were placed on a rack over an open dish of formaldehyde under a bell jar. The germinating spores were soon killed and the average lengths of the germ tubes, as based on measurements of 50 spores from each lot, are shown in table 3.

The results of these germination tests agree fairly well in temperature range with those of vegetative growth. The only fact worthy of note is that a markedly smaller percentage of spores of the large-spot form

TABLE 3.—*Germination of spores of celery Septorias at different temperatures for 14 hours*

Temperature (° C.)	Large-spot form		Small-spot form	
	Av. length of germ tube in μ	Germination	Av. length of germ tube in μ	Germination
		Per cent		Per cent
9 –12	3.1	14	10.1	82
11½–15	2.8	18	11.2	90
17 –19	6.9	26	23.7	94
20 –21	6.9	30	41.6	92
22 –23	11.1	28	43.3	94
23 –24	17.3	40	42.3	88
24 –25½	16.2	38	45.5	90
26 –27	14.7	26	27.6	60
27 –28	6.5	24	9.1	20
28½–30	1.0	12		
31 –34				
38 –42				

germinated, and the germ tubes were much shorter, indicating a longer incubation period. Campanile (10) did not get spore germination in sterile distilled water. It is quite possible that she worked with the large-spot form.

OXYGEN RELATION TO SPORE GERMINATION

Four capillary tubes were filled with a suspension of Septoria spores and sealed at each end, so that the liquid entirely filled the cavity of the tubes. Two other capillary tubes were filled and left open at each end. The tubes were placed in a culture room at 20–21°C. for 4 days. When the tubes were examined, there was no germination in the closed tubes, but the open tubes showed germination near the open ends.

OVERWINTERING STUDIES

The increased possibility of wide dissemination and early initial infection of both types of late blight makes the question of saprophytic growth on field soil very important. Two sets of 6 test-tubes each of sterile muck were inoculated by placing a single drop of a heavy spore suspension of each of the respective Septorias on the surface of the muck. In 2 more sets of 6 tubes, inoculations were made by transferring a small piece of agar containing mycelium. No growth, either macroscopic or microscopic, was observed in the tubes inoculated with the spore suspension. Only a few small filaments were found in the tubes in which the agar transfers were made, and these probably grew from the agar, as the soil did not support growth.

During the first week of April, 1930, partly decayed celery leaves from the previous year's crop, with definite lesions on them, were collected near Kalamazoo and Decatur, Michigan. The leaves examined in the laboratory revealed the presence of new and old pycnidia. A spore suspension obtained from the spots was sprayed on healthy celery plants and 14 days later the plants were badly affected with disease of the large-spot type. A similar collection, made in January of 1932, showed the presence of viable spores of the small-spot form on overwintered celery leaves.

LONGEVITY OF SPORES

The actual age at which Septoria spores lose their viability is not definitely known. Their longevity depends largely on storage conditions. Coons and Levin (9) recommend the use of 2- to 3-year-old celery seed in Michigan. Krout (17) found that conidia on celery leaves and on celery seed were dead in 8 to 11 months, and conidia in pycnidia on seed and pedicels failed to germinate after aging for two to three years. Flachs (13) reported the spores in pycnidia viable for one to two years. It was impossible for the writer to make tests of the longevity of spores on seed because of the uncertainty of the age of obtainable commercial seed.

Germination tests were made on spores from old specimens in the Michigan State College herbarium, but no viable spores were found. A specimen of the small type was collected in California and placed between a pair of ordinary blotters and sent to the writer through the mail. When the specimen arrived the spores did not germinate in agar and no infection resulted when the spores were sprayed on celery plants. One lot of leaves infected with the large-spot type was pressed between blotters and stored between sheets of newspaper in a drawer of a desk. After 8 months, plates were poured from these leaves and a considerable number of the spores germinated.

HOST RELATIONSHIP

Beach (3) found the members of the genus Septoria to be very limited in their host range. He made a few successful intergeneric inoculations in the same family but, in general, found members of the genus Septoria to be limited to a specific host and varieties of that host. Pethybridge (21) found a Septoria on wild celery that appeared very different from the one on cultivated celery, but upon cross inoculation the typical cultivated form developed. Thomas (28) tried the celery Septoria on several representatives of the Umbelliferae, with negative results. Laibach (18) tried his two forms of the celery organism on various members of the Umbelliferae, without success.

Since two separate species of Septoria are involved in this study, it was thought that one of them may have come from some nearly related umbelliferous host and, having found suitable environment, become pathogenic on celery. Seeds of various representatives of the Umbelliferae[3] were planted in sand and the seedlings were transferred to 3-in. pots, where they grew until large enough for inoculation. Separate lots of two plants of each of the species listed in the tabulation were atomized with a spore suspension obtained from typical leaf lesions of the two types of Septoria. Four healthy celery plants were atomized and placed in infection chambers with other representatives of the Umbelliferae as checks. The plants were left in the chambers for 52 hours after inoculation and then removed to the greenhouse bench, where the temperature was nearly 20°C. The following is a list of the Umbelliferae inoculated:

Aethusa cynapium L.
Ammi majus L.
Angelica purpurascens Lall.
Apium graveolens L. (Several lots)
" " " var. *rapaceum*
Apium repens Reicht.
Bowlesia tenera Spreng.
Bupleurum candollii Wall.
" *longicaule* Wall.
" *longifolium* L.
" *rotundifolium* L.
" *salicifolium* Soland.

[3] Seeds were sent to the writer from the following places: Missouri Botanic Garden, St. Louis; Royal Botanic Garden, Edinburgh; Museum d'Histoire Naturelle, Culture, Paris; Berlin Botanical Garden and Museum, Berlin; D. M. Ferry Seed Co., Detroit; Beal Botanic Garden, Michigan State College; and from local stores.

Carum carvi L.
Caucalis arvensis Huds.
" *nodosa* Gaertn.
Conium maculatum L.
Coriandrum sativum L.
Daucus carota L. var. Oxheart
" *grandiflora* Hoffm.
" *maximus* Desf.
Eryngium agavifolium Griseb.
" *amethystinum* L.
" *campestre* L.
" *heldreichii* Boiss.
" *planum* L.
" *wrightii* Gray
Ferula communis L.
" *glauca* Gouan.
Foeniculum vulgare Mill.
Heteromorpha aborescens Cham.
Levisticum officinale Koch.
Oenanthe crocata L.
" *lachenalii* Gmel.
" *peucedanifolia* Roll.
" *phellandrium* Lam.
" *pimpinelloides* L.
" *silaifolia* Bieb.
Opopanax chironium Koch.
Pastinaca sativa L. var. Danvers
Petroselinum sativum Hoffm.
" *segetum* Koch.
Peucedanum cervaria Cusson
" *graveolens* L.
" *officinale* L.
Pimpinella saxifraga L.
Scandix australis L.
" *balansae* Reut.
" *brachycarpa* Guss.
" *pecten-veneris* L.
" *pinnatifida* Vent.
Selinum tenuifolium Wall.
Seseli elatum L.
" *gummiferum* Poll.
" *libanotis* Koch.
" *tenuifolium* Ledet
Silaus flavescens Bernh.
Smyrnium olusatrum L.
Thapsia villosa L.
Thaspium aureum Nutt.
Tordylium maximum L.
Trachymene coerulea R. Grah.
" *pilosa* Sm.
" *pusillus* F. Muell.
Trinia kitaibelii Bieb.

Successful infection was obtained with both forms only on *Apium graveolens* and *A. graveolens* var. *rapaceum*. The check celery plants developed typical infection in every case. Infection was attempted on several members of the family Umbelliferae by placing small pieces of agar, containing fungus mycelium, on the leaves under absorbent cotton, but no typical disease was produced.

VARIETAL RESISTANCE

The following commercial varieties of celery were tested for evidence of resistance: Golden Self Blanching (tall strain), Golden Self Blanching (dwarf), Easy Blanching, M. A. C. Yellows Resistant A. A. 25, Simon's Golden Plume, White Plume, Burpee's Fordhook, Curly Leaf, and Giant Pascal. The easy-blanching varieties were all infected and soon lost their color and died, the plants affected with the large-spot form succumbing first. The green varieties seemed to be able to endure longer the effect of both Septoria species, but 10 days after the disease became evident, the plants affected with the large-spot form began to show yellowing near the edge of the leaves.

Thomas (28) reported the younger leaves of celery to be more susceptible to Septoria than the older ones, but less easily infected, due to less surface area. In infection experiments the writer found young and old

leaves infected with equal ease. This tends to show that infection is more a matter of contact, or ability of the fungi to gain entrance, than susceptibility.

MODE OF INFECTION

Campanile (10), working with what is probably the large-spot form, described the fungus as entering the celery leaf by penetrating directly through the walls of the epidermal cells. After the fungus was established in the epidermal cells, it grew into the intercellular spaces and a characteristic spot was produced by collapse of the tissue.

Spores of both forms were placed in drops of water on the lower epidermis of celery leaves that had been removed and placed in a moist chamber. The epidermis was stripped from the leaf at different intervals and the progress of germination of the spores observed. After 3 days germ tubes were formed from various cells of the spores (Fig. 3, C to G). The first noticeable change previous to germination was the appearance of large oil droplets in the spores. The spores of the small type showed a markedly greater accumulation of oil. Spores of both types of Septoria showed an increase in the number of septa just previous to germination. In many cases the germ tubes were observed to grow directly across the surface of a stoma without entering it (Fig. 3, H, I). A single case was observed where the small slender germ tube of a spore of the small-spot type entered an epidermal cell near its anticlinal wall and branched inside the cell (Fig. 3, K).

Inoculated leaves that had been kept in a moist chamber for a period of 4 days were examined. Small hyphal systems had been established in several cells that were not bordered by stomata. Leaves inoculated on different surfaces showed the same number of spots per leaf, although the ratio of stomata per field under low power of the microscope was for upper to lower surfaces as 3.4 is to 8.2.

These observations have led the writer to believe that entrance may be through any portion of the surface of the leaf. There seems to be no stimulus that attracts the germ tubes to the stomata. Entrance through a stoma is possible, but the fungus may enter a guard cell, as well.

LEAF-STALK INFECTION

Dorogin (11) recognized typical lesions produced on the leaf stalks by the small-spot form but did not find lesions of his large-spot form on the leaf stalks. Foster and Weber (14) described their two strains of the celery Septoria as producing identical lesions on leaf stalks.

Portions of leaf stalks were washed in sterile water and placed in a sterile moist chamber. Drops of spore suspensions of each form of the Septorias were spread along different stalks. After 16 days typical lesions developed on the stalks inoculated with the small-spot form. The stalks

inoculated with the large-spot form developed very shallow brown spots, but no pycnidia were formed.

No lesions on leaf stalks were found in any of the infection experiments with the large-spot type of Septoria on celery in the greenhouse, and no such lesions were observed as the result of natural infection in the field. Lots of market celery were atomized with spore suspensions and stored at room temperature. A few lesions of the small-spot type developed, but none of the large-spot type was noted.

FUSING OF GERM TUBES

Another feature noted in culture and on leaves was the early fusing of germ tubes of germinating spores (Fig. 3, D, G, H, J). The germ tube from an occasional spore seemed to grow in a given direction until in proximity to another, when it turned shortly and fused as if attracted by some force. An occasional spore would form a single germ tube that would fuse directly with that of a similar spore, while none of the other cells in the spore germinated. If conidia are merely encysted vegetative portions of an organism, it would be only natural to expect that the germinating conidia would fuse as do other hyphae in culture. Time did not permit inoculation experiments with single and with fused conidia.

EFFECT OF LIGHT ON PYCNIDIUM FORMATION

Although pycnidia may be found on both sides of a leaf, it was observed in natural infection that they were most plentiful on the best illuminated side. To study this factor, healthy celery leaves were placed in a large glass culture dish. In a series of leaves inoculated with a drop of spore suspension on the normal top side, some were placed with the upper side and some with the under side up. A similar set-up was made with the leaves inoculated on the under side. In all cases the greatest number of pycnidia were found on the side toward the light.

GEOGRAPHIC DISTRIBUTION OF THE TWO SPECIES OF SEPTORIA

Immediately after it had been proved definitely that there were two species of Septoria attacking celery, the question of geographic distribution arose. Septoria on celery has been reported from all parts of the United States where the crop is grown, but, since only one form was recognized, the same name has been applied to both forms. Since only the small-spot form has been found on celery leaf stalks, the damage reported on celery in storage was most likely due to it. Some of the damage to celery has been accredited to *Septoria petroselini* Desm. It has been proved that the celery forms do not attack parsley, but lack of material prohibited a test of the parsley Septoria on celery.

From literature, specimens, and correspondence, it seems that the large-spot Septoria is the common form on celery in Europe. Although the

large-spot form was observed in the United States about the same time as it was in Europe, the small-spot form is the most common and destructive at the present time in the United States.

The distribution of the two forms in the United States may be indicated by the data in table 4, which is the result of a questionnaire sent to the States where celery is grown and examination of the specimens received.

TABLE 4.—*Distribution of the two forms in the major celery-growing regions in the United States*

State	Material examined	Occurrence	
		Small type	Large type
California	4 specimens	3 specimens	1 specimen
Oregon	4 "	2 "	2 specimens
Washington	3 "	2 "	2 "
Minnesota	3 "	1 "	2 "
Wisconsin	letter and 2 specimens	most prevalent 1 specimen	widespread 1 specimen
Indiana	personal observation	most prevalent	has been seen
Michigan	personal observation and 2 specimens	" " 2 specimens	common
Ohio	5 specimens	5 "	
New York	8 "	6 "	2 specimens
Delaware	3 "	1 "	2 "
Massachusetts	7 "	7 "	2 "
New Jersey	3 "	1 "	2 "
Florida	2 " and Fla. Sta Bul. 173.	1 " widespread	2 " widespread

NAMES OF THE CAUSAL ORGANISMS

The actual date when Septoria was first described on celery is somewhat disputed and uncertain, due to the fact that several workers described fungi resembling Septoria at about the same time. Spegazzini (24) in 1887 described a fungus on *Apium australe* from Tierra del Fuego and Staten Island under the name *Septoria apiicola*. Although this is the first record of a Septoria on Apium, it is impossible from the description to tell whether the fungus is identical with either of the two on cultivated celery. It is possible that, upon examination of specimens of the fungus on *A. australe* and after cultural and inoculation studies, this may prove to be the same as one of the celery Septoria species, but, until such studies can be made, it seems advisable to maintain it as a separate species. Briosi and Cavara, in 1890, described a fungus on celery in Italy, which they

called *Septoria petroselini* Desm. var. *apii*. They collected and distributed it with descriptions as No. 144 in *I Funghi Parassiti*. They retained the specific name because of its likeness to the Septoria on parsley; yet, by the varietal name, indicated the difference between the celery and the parsley organisms.

At about the same time Allescher collected specimens of a fungus on celery in Bavaria, which he sent to Bresadola. Allescher, in his letter, called the fungus *Septoria Magnusiana*, but in describing the fungus, Bresadola noted some subhysteroid ostioles and apparently imperfect pycnidia and transferred the fungus from Septoria to Phlyctaena. These specimens collected by Allescher were distributed under Bresadola's name *Phlyctaena magnusiana* (Allesch.). Bres. as No. 188 of Allescher et Schnable, *Fungi Bavarici*. This set was dated 1890 but was not distributed until 1891. Accompanying the specimen No. 188, there was a printed description of the new species, so that the publication of the specific name *magnusiana* dates from early in 1891, when the set was distributed. The species description was published by Allescher (1) in 1892.

Chester (4, 5), in Delaware in 1891, noted the presence of the fungus on celery and identified it with *Septoria petroselini* Desm. var *apii*. Br. and Cav. He compared the form on celery with the true *S. petroselini* Desm. on parsley and decided that the differences were sufficient to warrant raising the variety *apii* to specific rank. He published the name *S. apii* (Br. et Cav.) Chester (5) in the bulletin of the Torrey Botanical Club, Dec. 9, 1891. According to Klebahn (16), Rostrup in 1893, ignorant of Chester's work, also described the fungus under the name *Septoria apii* Rostrup.

The name *magnusiana* is the first specific name applied to the fungus and according to the rules of nomenclature, would be the correct name to use, except for the fact that Allescher subsequently, in 1892, used the combination *Septoria magnusian* Allescher for an entirely different species on *Spiraea chamaedryfolia*. Thus, the earliest available name for the celery organism is *Septoria apii* (Br. and Cav.) Chester.

According to Klebahn (16) and Dorogin (11), who examined the specimens in the sets of exsiccata, *Septoria petroselini* Desm. var. *apii* Br. and Cav. is identical with *Phlyctaena magnusiana* (Allesch.) Bresadola. The present writer has compared Chester's original specimens (sent through the kindness of Dr. Manns) with those of Briosi and and Cavara and confirms Chester's findings.

From the description and specimens of Briosi and Cavara, Klebahn, and Chester it is clear that the fungus studied by them is identical with the large-spot type studied by the writer. Although the specimens of Briosi and Cavara are identical with the large-spot type, the descripion accompanying the specimens does not exactly fit the specimens. The pycnidia

are described as crowded and fusing. It seems quite possible that both types of Septoria may have been present in Italy and the small-spot type may have confused the description, the author not knowing that there were two separate Septorias attacking celery. The name *Septoria apii* (Br. and Cav.) Chester then applies to the causal organism of the large-spot type of the disease.

In 1915 Dorogin (11) described two distinct Septorias as parasites on celery. One type he described as causing a large spot, reddish brown, and, when mature, having minute pycnidia sparsely scattered near the center of the spot. The pycnidia were nearly round, paraplectenchymatous in nature, 70 – 125μ in diameter; the ostiole $\frac{1}{3}$ to $\frac{1}{4}$ the diameter of the pycnidium and round or irregular in form. The spores were filiform, 30 to 40 μ × 1.5 to 2 μ, about straight without visible granules, and with 3 inconspicuous septa. No pycnidia were found on the stalks. This form he identified with the Briosi and Cavara and Chester type, *i.e., Septoria apii* (Br. and Cav.) Chester. The other he described as causing a small spot with densely crowded, often fused, black pycnidia. The pycnidia were 90 to 135 μ in diameter, paraplectenchymatous in nature and characterized by very large ostioles often somewhat slit and sunken in the uncollapsed tissue around the spot. The spores were 45 – 50 × 2 – 3.5 μ, obtuse at the ends, distinctly granular and as many as 6-septate. Lesions with crowded pycnidia were abundant on the leaf stalks. He concluded that this second fungus was a new species and gave it the name *Septoria apii-graveolentis.*

Laibach (18) in 1921, in reviewing the work of Dorogin and reporting his own work, considered the two types of the celery late-blight organisms as varieties of the species *Septoria apii.* He gave the names *S. apii* var. *maculiformis* and *S. apii* var. *punctiformis* to the large- and small-spot types, respectively. His reason for this was that he observed saltations in his cultures in which both forms were represented. From pictures of the disease on leaves and pictures of the cultures in Petri dishes, it appears that the two fungi he described are similar to those of this study. However, since pure cultures of the organisms showed no such saltation and always produced typical forms of the disease for the writer, and the spores are quite different in size and shape, and the physiological reactions of the organisms are markedly different, the writer agrees with Dorogin in regarding the two forms as separate species.

One specimen mentioned by Dorogin as examined and found identical with his *Septoria apii-graveolentis* was collected in London, Ontario, Canada, by Dearness. A specimen of this same collection (Fungi Columbiani) in the herbarium of Michigan State College was found to be identical with the small-spot type occurring in Michigan. The name of the causal organism of the small-spot type then should be *Septoria apii-graveolentis* Dorogin.

SUMMARY

1. Two distinct species of Septoria were isolated from late-blight lesions on celery and definitely proved to cause different types of disease.

2. The two diseases on celery are characterized by large and small lesions and may be distinguished accordingly. The large-spot lesion is regular in outline and has small, black, nearly spherical pycnidia scattered singly in its central portion. The small-spot lesion is indefinite in outline and has pycnidia of nearly twice the size of those of the large-spot type. The pycnidia of the small type may be found in both collapsed and uncollapsed tissue, occurring singly or fused in twos and threes. Fruiting bodies are never found in the large-type spot until a definite region of tissue has collapsed and even then only under conditions of moderate to high humidity.

3. In pure culture the two species of Septoria grew well on most laboratory media, but the type of growth was characteristically different. The large-spot organism made a spreading growth with black submerged mycelium and white aerial mycelium. The small-spot organism made a more tufted growth of somewhat cheesy consistency. On potato-dextrose agar, the large-spot organism produced a purple color in the medium around the colony.

4. Spores of the small- and large-spot organisms were killed in 10 minutes at 41° C. and 43° C., respectively. The optimum temperature for growth of the small-spot fungus was between 18° and 22° C., while that for the large-spot one was between 22° and 24° C.

5. A large number of the family Umbelliferae were inoculated with both fungi, but infection resulted only on *Apium graveolens* (celery) and its variety *rapaceum* (celeriac).

6. Several varieties of commercial celery were inoculated and, although the green celeries seemed more able to endure the diseases, all seemed to be equally susceptible.

7. Although both species of Septoria are widely distributed in the United States, the name generally used in literature and applied to specimens in herbaria is that of the large-spot organism. Geographic studies showed that the fungus causing the large spot is probably the common one in Europe, but the small-spot-producing organism is far more common in this country.

8. The small-spot type of disease is caused by *Septoria apii-graveolentis* Dorogin. The large-spot organism is *Septoria apii* (Br. and Cav.) Chester.

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SOME ENVIRONMENTAL RELATIONS OF WATERMELON WILT

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INTRODUCTION

The intimate relation of such variables as temperature, moisture, and soil reaction, sometimes termed "conditioning factors," to the occurrence and severity of certain plant diseases is well recognized. Fluctuations among these factors doubtless exert either direct or indirect influences on both the host and parasite. While the effect of each variable probably never can be completely segregated and while the environmental conditions under which plants are grown experimentally cannot be exactly duplicated under field conditions, yet the results as reported by various workers have been remarkably consistent with respect to the behavior of the diseased plant when subjected to different environmental conditions.

It is the purpose of this paper to set forth indicative evidence relative to the effects of such conditioning factors as air temperature, air humidity, wind velocity, and sunlight intensity on the rate of death of watermelon plants growing in wilt-infested soil. Taken collectively, these effects were measured by the rate of water evaporation from atmometer cells. Taken singly, some effects of all except wind velocity are indicated. These studies were made in Iowa in 1927 and 1928 and at Davis, California, in 1931.

HOST RESPONSE

Watermelon wilt, the vascular disease caused by *Fusarium niveum* E. F. Sm., is manifested by slightly different host response from that of vascular fusarioses of tomato, cabbage, cowpea, and cotton. With the latter plants, death does not always quickly follow the first manifestation of infection, but diseased plants may cease to manifest symptoms, partially recover, or even reach maturity before the plants die. A wilt-infected watermelon plant usually dies within 10 days after the first sign of disease becomes evident. In many cases infected plants die within 24 to 48 hours after wilting begins. Seedling plants may succumb with 2 to 6 hours after the cotyledons begin to droop. While the reasons for this diversity of host response are not known, it is suspected that the greater leaf area of the watermelon plant, with consequent rapid and extensive water loss might offer a partial explanation. Furthermore, the more woody nature

[1] The writer gratefully acknowledges the assistance of Dr. H. A. Jones and Dr. J. B. Kendrick in preparing this paper for publication.

of such plants as tomato, cotton, sweet potato, and cabbage may influence their relatively slow rate of death following infection.

Observations indicated that with an abrupt rise of air temperature in the field, the rate of death in watermelons increased. Counts made at 6:00 A. M. following a cool night, compared with similar counts following a warm night, showed that in the former case infected plants had often temporarily recovered. In the latter case, infected plants were dead. Was recovery due to decreased transpiration from the infected plant or was it the result of low temperature effects on the pathogen? When watermelons were planted in infested soil during cool weather, seedling wilt was of rare occurrence. If the weather remained cool for several days following emergence, wilting was correspondingly retarded. When, however, planting was delayed until the soil became warm and when a period of warm weather followed emergence, seedling wilt was rapid. Was this entirely due to temperature effects on the host and pathogen or could it be correlated with the rate of evaporation from the leaves? The temperature relations are known (8) and investigation has shown (9) the relation of soil temperature to rate of wilting and manifestation of symptoms under controlled greenhouse conditions, but critical examination of interrelated environmental effects in the field has not been made.

RELATED WORK

The literature on the relation of environmental factors to the occurrence and severity of fusarial wilts has been reviewed so often of recent years that further discussion is unnecessary. Attention is directed to a few papers in which more detailed discussion may be found. There is an extensive paper by Jones *et al.* (6), dealing with the relations of soil temperature to several plant diseases. There are also papers by other workers as follows: Gilman (3), Tims (11), and Walker and Smith (13) on cabbage yellows; Clayton (1, 2) on tomato wilt; Harter and Whitney (4) on sweet-potato stem rot; Linford (7) on pea wilt; Tisdale (12) and Jones and Tisdale (5) on flax wilt; Young (14) on cotton wilt; and by Porter and Melhus (10) on watermelon wilt. In general, it may be stated that the vascular fusarial diseases are probably limited in their geographical distribution and seasonal severity by low soil temperature.

METHODS AND MATERIALS

Soil. The soil used in the trials in Iowa and California was known to be heavily infested with *Fusarium niveum*, as previous watermelon crops of commercial varieties had been almost a complete failure because of wilt. The soil used in California was of a heavier type and contained much less sand than the soil in Iowa. Artificial irrigation was practiced only in California.

Seed. For the trials in Iowa, seed of 4 commercial varieties was used, namely, Kleckley Sweet, Halbert Honey, Tom Watson, and Thurmond Grey. In the California trials, seed of the commercial variety Klondike was used. All seed was disinfected before planting.

Culture. In Iowa, the seed was planted in rows 6 ft. apart with approximately 4 ft. between the hills in the row and at the rate of approximately 15 seeds per hill. Seed was planted on top of a ridge under which commercial fertilizer and manure has been applied. In California, seed was sown on raised beds with a furrow between to facilitate irrigation. The seeds were planted approximately 2 in. apart, planting being purposely delayed until May 15 so that the soil temperature would be at or near optimum for growth of the causal organism and infection of the plants. Previous experience indicated that many of the plants would die before producing the 4th leaf, hence close planting would not result in crowding of the plants. The land was irrigated 2 days before planting and daily during the course of the experiment.

Standard spherical atmometers were used for measuring the rate of evaporation. Four cells, 2 white and 2 blackened with a mixture of lamp black, collodion, and ether were used at Davis. The bottles that supported the atmometers were placed in a hole in the ground so that the cells would be near the soil surface at approximately the same elevation as the watermelon foliage. Distilled water was used and the readings of water lost were made at 7:00 A. M. every day from May 24 to June 25, inclusive. Each morning the white cells were washed and the black cells gently rubbed with cotton moistened with distilled water. Twice during the period indicated the black cells were recoated with the lamp-black mixture. Similar apparatus was used in the Iowa experiments, but a mixture of lamp black and water, instead of the collodion mixture, was used to blacken the cells.

Six separate trials were conducted in Iowa from June 23 to September 3. The trials differed from one another in location of the fields, age of the plants, time of the year, and length of the period considered. Trial No. 1 included 4 periods of 96 hours' duration, each; No. 4 included 3 periods; while trials Nos. 2, 3, 5, and 6 were divided into 2 periods, each. In general, seedlings were not considered, the counts being based on the rate of wilting in older plants. Stand counts were made at regular intervals and as the atmometers were read daily it was possible to determine the average daily rate of evaporation for any period desired. Whenever stand counts were made, wilted plants were pulled and discarded.

One test, divided into 16 periods, was conducted in California. At the time of daily readings of the atmometers, the total number of plants was counted and the wilted plants were pulled and discarded. The percentage of wilted plants was determined from the daily counts.

RESULTS

Iowa Tests. The results of the tests in Iowa appear in table 1. With each of the 15 periods considered, the white atmometer evaporation, B-W,[2] and percentage of plants wilted are indicated. In addition, the length of each period, with inclusive dates, is shown and the maximum and minimum air temperature indicated. Counts made before June 23 were not considered, and the soil temperature after that date was at or near the optimum for fungus growth and infection.

Test No. 1 (periods 1 to 4, inclusive). This test continued from June 23 to July 8, inclusive, each period being of 96 hours' duration. The average percentage of wilt and evaporation rate, per 24 hours, was determined by dividing the respective totals for each period by 4. By standardizing the period duration at 24 hours in the Iowa tests, the data are summarized in one table rather than in six. Neither the rate of wilting nor the rate of evaporation varied greatly during test 1, but during period 2 both evaporation and wilting rates were higher than during the other 3 periods and the maximum and minimum air temperatures were higher than during periods 1 and 3.

Test No. 2 (periods 5 and 6). This test was divided into 2 periods of 168 hours, each, and extended from June 27 to July 10, inclusive. Both evaporation and rate of wilting were lower during the latter period, with no appreciable difference in maximum and minimum air temperatures per period.

Test No. 3 (periods 7 and 8). This test continued from July 17 to 28, inclusive. The evaporation rate was higher but the wilting rate was lower during period 7 than during 8. This is the only case in the 6 tests of apparent negative correlation. As the 2 periods were of unequal duration, such might be the explanation. It should be noted, however, that the value of B-W and the maximum and minimum temperatures were higher during the latter period.

Test No. 4. This test was divided into periods 9, 10, and 11 and extended from July 17 to 27, inclusive. By comparing evaporation with wilting rates during successive periods, it is evident that any marked change in evaporation rate was accompanied by a similar change in rate of wilting. Both evaporation and wilting rates were much lower during period 10 than during 9, but during period 11 the evaporation rate increased over period 10 with a corresponding increase in rate of wilting. Similar comparisons are evident between wilting rate and B-W.

[2] The difference in rate of evaporation between the black and white atmometer is indicated as "B-W." This value is a measure of the relative light intensity.

TABLE 1.—*The relation of rate of evaporation and sunlight intensity to the rate of watermelon wilting due to infection with Fusarium niveum. Date from tests conducted in Iowa in 1927*

Test No.	Period			Average evaporation in cc. per 24 hours of each period		Average percentage wilted plants per 24 hours of each period	Air temperature in degrees C.	
	No.	Hours' duration	Inclusive dates	From white atmometers	B-W[a]		Maximum	Minimum
1	1	96	June 23–26	39.8	16.1	1.3	30	14
	2	96	" 27–30	60.5	21.7	2.5	35	18
	3	96	July 1–4	34.3	17.8	1.1	30	13
	4	96	" 5–8	39.7	20.0	1.8	35	18
2	5	168	June 27–July 3	51.6	19.3	2.4	35	13
	6	168	July 4–10	37.3	18.2	1.3	35	13
3	7	192	July 17–24	26.3	12.7	2.6	33	13
	8	96	" 25–28	18.7	20.1	3.8	35	17
4	9	72	July 17–19	41.3	24.8	4.5	33	13
	10	96	" 20–23	10.3	11.5	1.4	32	13
	11	96	" 24–27	18.5	20.1	9.6	35	17
5	12	72	Aug. 28–30	27.5	11.2	6.5	31	9
	13	72	" 31–Sept. 2	32.2	17.4	15.2	34	16
6	14	48	Aug. 30–31	28.4	15.3	4.9	33	13
	15	48	Sept. 1–2	35.3	20.6	9.4	34	17

[a] The difference in rate of evaporation between the black and the white atmometers is indicated as B-W. This value is a measure of relative light intensity.

Test No. 5 (periods 12 and 13). This test began on August 28 and terminated on September 2. Rate of wilting as well as the evaporation rate was more rapid during the latter period. Similar data are presented for test No. 6 (periods 14 and 15), which extended from August 30 to September 2. While there was a constant relation between evaporation and wilting rates during tests 5 and 6, there was also a constant relation between wilting rate and B-W.

It should again be pointed out that the 6 tests are not comparable with each other, as they were conducted in different fields, at different dates, and with plants of varying age. Within each test only successive periods are comparable. The important consideration involves the effect of a marked change in evaporation upon the rate of wilting. The relation of evaporation to rate of wilting is indicated graphically in figure 1, A, while the relation of B-W to rate of wilting appears in figure 1, B.

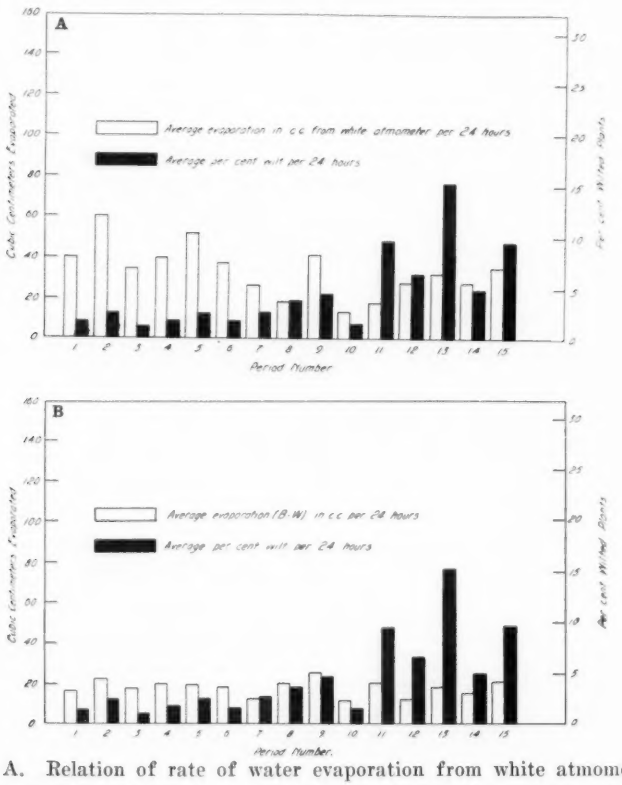
A
Average evaporation in c.c. from white atmometer per 24 hours
Average per cent wilt per 24 hours
Cubic Centimeters Evaporated
Per cent Wilted Plants
Period Number
B
Average evaporation (B-W) in c.c. per 24 hours
Average per cent wilt per 24 hours
Cubic Centimeters Evaporated
Per cent Wilted Plants
Period Number

FIG. 1. A. Relation of rate of water evaporation from white atmometer cells to the rate of watermelon wilting due to infection with *Fusarium niveum*. It is possible to compare only 2 successive periods for reasons stated in the text. B. Relation of sunlight intensity to rate of wilting. Sunlight intensity was determined from the difference in evaporation rates between black and white atmometers. All data taken from table 1 (Iowa trials, 1927).

While the periods are too few in number to attempt statistical correlation, the constant tendency for relatively rapid wilting to accompany periods of relatively high evaporation rate indicates that positive correlation exists. The application of statistical formulae to environmental effects that are influenced by many separate conditioning factors might not be so accurate as data that show merely a consistent tendency in one direction.

California Tests. Data derived from the tests in California are tabulated in 16 periods of 48 hours, each, beginning May 23 and terminating on June 23. Unlike the Iowa tests, seedlings were mainly considered, although, by June 23, many of the plants had just started to form runners. In addition to the average evaporation from both black and white cells, the following records were also secured: the average soil temperature at depths of ½, 3, 6, and 12 in.; the relative humidity of the air at 12:00 A. M.; and the mean, maximum, and minimum air temperatures. These data are presented in table 2.

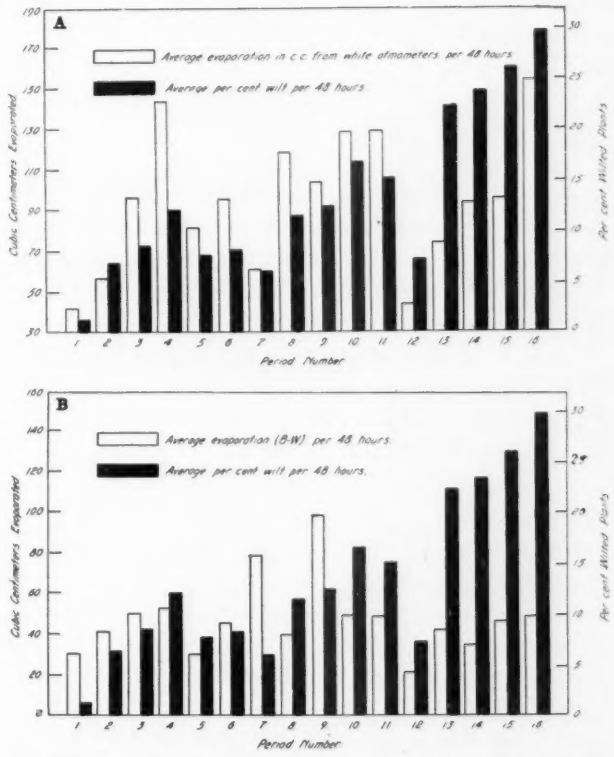
A
Average evaporation in c.c. from white atmometers per 48 hours.
Average per cent wilt per 48 hours.
Cubic Centimeters Evaporated
Per cent Wilted Plants
Period Number
B
Average evaporation (B.W.) per 48 hours.
Average per cent wilt per 48 hours.
Cubic Centimeters Evaporated
Per cent Wilted Plants
Period Number

FIG. 2. A. Relation of rate of evaporation from atmometer cells to rate of wilting. B. Relation of sunlight intensity to wilting. All data taken from table 2 (California trials, 1931).

TABLE 2.—*The relation of rate of evaporation and sunlight intensity to the rate of watermelon wilting due to infection with Fusarium niveum. Data from tests conducted at Davis, California, in 1931*

Period		Average evaporation in cc. from 1 cell			Percentage wilted plants	Average soil temperature in degrees C.				Air temperature in degrees C.			Relative humidity at 12:00 A. M. average for 2 days
No.	Inclusive dates	From black cell	From white cell	B-W[a]		½″	3″	6″	12″	Mean	Maximum	Minimum	
1	May 23–24	73	42	31	1.2	.....	.....	.....	.....	15	28	13	52.5
2	25–26	99	57	42	6.4	.....	.....	.....	.....	16	26	10	62.0
3	27–28	148	97	51	8.6	.....	.....	.....	.....	23	37	11	51.5
4	29–30	197	144	53	12.1	28	28	27	26	23	38	11	30.5
5	31–June 1	113	82	31	7.8	26	28	26	26	15	31	10	42.0
6	2–3	143	96	47	8.2	26	25	25	25	16	28	9	50.0
7	4–5	141	62	79	6.1	28	26	26	25	23	34	11	37.5
8	6–7	159	118	41	11.5	27	26	26	25	20	31	11	46.0
9	8–9	202	104	98	12.4	27	27	26	25	17	28	10	47.5
10	10–11	176	128	48	16.6	29	28	27	26	19	32	10	39.0
11	12–13	178	129	49	15.1	29	29	28	27	20	34	11	36.0
12	14–15	66	44	22	7.2	24	23	24	25	18	24	11	60.5
13	16–17	115	73	42	22.2	24	24	24	24	16	29	8	47.0
14	18–19	128	93	35	23.3	25	26	25	24	18	28	11	40.0
15	20–21	143	96	47	26.1	28	27	27	25	19	33	11	42.0
16	22–23	202	154	48	29.7	28	28	28	27	23	33	9	30.0

[a] Indicates relative sunlight intensity.

During 15 of the periods, when the rate of evaporation increased there was an increase in rate of wilting. During period 1 the average evaporation was 42 cc. with only 1.2 per cent wilt, but during period 2 the average evaporation was 57 cc. with 6.4 per cent wilt. Similar relations are apparent when comparing periods 4 with 5, 7 with 8, 11 with 12, 12 with 13, and 15 with 16. Correlation of white-cell evaporation rate with rate of wilting is clearly evident from the graphical representation in figure 2, A, when successive periods are compared.

The relation between rate of wilting and sunlight intensity, represented in table 2 and figure 2, B, as B-W, is also well expressed. During 10 of the 16 periods positive correlation apparently existed. In most cases periods of increased sunlight intensity were accompanied by more rapid wilting than during the preceding or succeeding period of less intense sunlight. During period 11 the value of B-W was 49 cc. and the percentage of wilt was 15.1, but during period 12 the value of B-W decreased to 22 cc. and the percentage of wilt to 7.2. Similar relations are apparent when comparing periods 2 with 3, 4 with 5, 12 with 13, and 14 with 15.

AIR TEMPERATURE

A brief statement of the relation of air temperature to rate of wilting ing the field was reported previously by the writer (9). The following data and discussion constitute a more detailed report.

The relation of air temperature to the rate of wilting was studied in 2 infested fields at Conesville, Iowa, in 1927 and 1928 and in a field at Davis, California, in 1931. The soil used was so heavily infested with the wilt fungus that commercial crops suffered more than 99 per cent mortality. In Iowa, disinfected seed of the varieties Kleckley Sweet and Tom Watson was planted at intervals from May 20 to July 1, stand counts being made from the date of emergence until the end of the growing season. The difference in the number of living plants at the time of succeeding stand counts was assumed to be due to wilt. The percentage of wilt thus computed was then divided by the number of days that had elapsed since the last reading giving "average per cent daily wilt since last reading."

The air temperature was recorded automatically by a Friez thermograph. The total number of hours that the air temperature was above a certain point was determined for the interval between the dates of successive stand counts. This number was then divided by the number of days that had elapsed since the previous reading, giving the average number of hours per day that the air temperature was above 24°, 27°, 29°, 32°, and 35° C.

The relation between air temperature and rate of wilting is presented graphically in figure 3, considering only the relation existing at tempera-

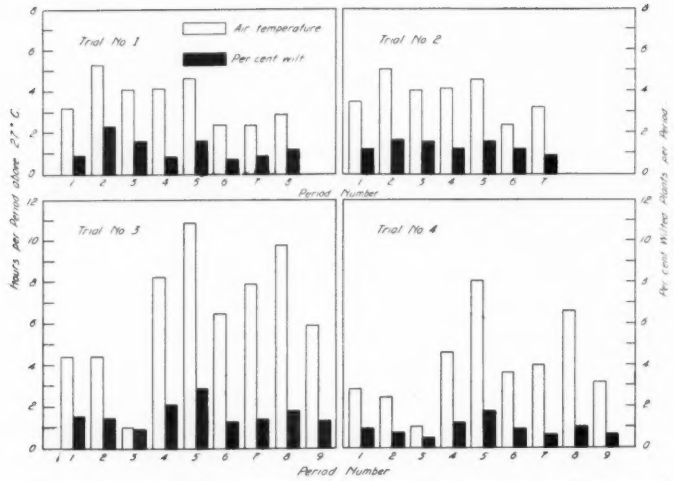

FIG. 3. The relation of air temperature above 27° C. to rate of watermelon wilting due to infection with *Fusarium niveum*. (Similar data are available with respect to temperatures of 24°, 29°, and 32° C.) Only successive periods of each trial may be compared. The tendency is for rapid rate of wilting to parallel sustained periods of warm weather under field conditions.

tures at or above 27° C. Similar charts were prepared for 24°, 29°, and 32° C., and, as the same general tendency was evident, only one chart is included herewith. It should be stated that the nature of the data is such that different tests should not be compared, the reason being that each test extended over a month or more. Comparisons should be made only between 2 successive periods of the same test. Considering test 1; the percentage of wilted plants during periods 1 and 2, respectively, was 0.9 and 2.4; at the same time the air temperature was above 27° C. for 3.3 hours during period 1 and for 5.5 hours during period 2. During period 3, the percentage of wilted plants decreased to 1.8 and the total hours above 27° C. to 4.2.

In test 2 the relation seems somewhat less evident than in test 1, but there is the same general tendency. A striking correlation is apparent when subsequent periods of test 3 are considered. The abrupt and sustained rise in air temperature during period 4 and accompanied by an increase in the rate of wilting from 0.9 per cent during period 3 to 2.1 per cent during period 4. Even warmer weather prevailed during period 5 than during period 4, with an accompanying increase in the rate of wilting. During period 6, cooler weather than during period 5 prevailed and rate of wilting decreased. Parallel instances are also evident during trial 4, particularly when period 3 is compared with 4, 5 with 6, 7 with 8, and 8 with 9.

In general, the data indicate that with relative increase in the number of hours of warm weather the rate of wilting increases. It is not known whether this increase in wilting rate is due to abrupt rise in air temperature or to sustained high temperature. An abrupt rise in temperature would lower the relative humidity, since warmer air can hold more water. This would favor wilting. When the 32-degree chart was examined it was apparent that the relation was very similar to that expressed for 27° C. in figure 3. Obviously, during the same period, the air temperature would be above 32° C. for a much shorter time than above 27° C., yet the relations to wilting rate were approximately the same. It is possible that either an abrupt weather change or a sustained period of changed weather conditions would induce like effects. In addition, the relations seem to hold whether seedlings or more mature plants were considered.

In California, air temperature, soil temperature, and relative humidity were determined during the period that the evaporation rate was being measured. The relation of these factors to the rate of wilting is indicated in table 2, attention being directed particularly to the relations between the mean air temperature and the rate of wilting when successive periods are compared. It appears that during 10 of the 16 periods correlation is positive. These findings agree with those of the Iowa tests. As the correlation between rate of wilting and rate of evaporation from white cells (Fig. 2, A) is much closer than that from mean air temperature (Table 2), it is evident that such additional conditioning factors as wind velocity, air humidity, and sunlight intensity exert some influence. The data on the effects of sunlight, indicated as B-W, are included in table 2 and in figure 2, B. The humidity relations indicated in table 2 are less conclusive, but association of low humidity with high wilting rate was evident in 8 of the 16 periods.

It should be noted that the average soil temperature per period at depths of ½, 3, 6, and 12 in. was never below 74.5° F., and, as infection may occur at or above 68° F., it may be concluded that the average soil temperature during the course of the experiment was such that infection could take place.

DISCUSSION

The investigations herewith reported were not conducted under controlled conditions; they were conducted in the field in an attempt to measure the cumulative effects of variation in natural environmental conditions on the rate of death of watermelon plants growing in soil heavily infested with *Fusarium niveum*. While it is recognized that more positive conclusions result when environmental conditions are controlled, yet the results presented herewith indicate what happened under field conditions.

In general, those external conditions of environment that determine the rate of transpiration from leaves and the rate of evaporation from atmometer cells appear to influence the rate of wilting of watermelon plants infected with *Fusarium niveum*. In these experiments, the soil-moisture supply was adequate for noninfected and for resistant plants; thus deficient moisture supply alone was not responsible for wilting. Whether infected plants were able to absorb sufficient water from the soil is not known; furthermore, the transpiration rate from them was not determined. Working with pea wilt (*F. orthoceras* var. *pisi*), Linford (7) presented some evidence that wilting resulted from excessive water loss from the leaves of infected plants, and not entirely from a diminished water supply.

The data herewith presented indicate that variation in the rate of wilting depends to some extent upon the variations in those conditioning factors that influence transpiration. Combinations of increased light intensity, abrupt or sustained rise in air temperature, and low relative humidity increase the rate of transpiration. The data indicate that these conditioning factors exert similar effects on rate of wilting. It seems evident, therefore, that rate of wilting is determined by the extent of interference with water movement in the plant. These experiments support the theory that the presence of the fungus in the conducting tissues interferes with water movement. It is not known whether this interference is due to mechanical obstruction by the mycelium and spores, to indirect influences of the pathogen, or to a chemical substance formed by the fungus.

SUMMARY

The rate of watermelon wilting due to infection with *Fusarium niveum* is influenced by such environmental factors as air temperature, relative humidity, sunlight intensity, and wind velocity.

Tests conducted in Iowa in 1927 and 1928 showed that there is a tendency toward positive correlation of the air temperature with the rate of wilting. Similar conclusions are drawn from the results of trials conducted in California in 1931.

Sunlight intensity also seems to have some relation to the rate of wilting, the tendency being for relatively rapid wilting to follow periods of relatively intense sunlight as determined by the difference in the rate of evaporation between the black and white atmometer cells. Although this rate was much higher in California than in Iowa, the same general conclusions may be drawn. From the California tests it is concluded that the rate of wilting increased with a decrease in the relative humidity of the air.

The rate of evaporation as indicated by white atmometer cells, influenced by air temperature, air humidity, light intensity, and wind velocity, ap-

pears to influence the rate of wilting. With increase of evaporation rate, the rate of wilting increases. Relatively slow rate of wilting occurs during periods of relatively slow rate of evaporation.

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RHIZOCTONIA SOLANI ON CEREALS IN SOUTH AUSTRALIA

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(Accepted for publication March 7, 1932)

Although *Rhizoctonia solani* Kühn is known to have a very wide host range and has been reported by Rayllo (5) as affecting wheat and oats in cross-inoculation studies with the potato strain of the organism, yet so far this fungus does not seem to have been reported as causing a definite disease of cereals in the field. It is true that Rhizoctonia has been identified, though usually as one of a number of fungi, in isolations from foot rots of wheat in several countries. In these cases, however, the disease has not always been definitely attributable to the Rhizoctonia, and the identification of the fungus has usually not been carried so far as the species. One of the most definite cases on record appears to be the occurrence of a Rhizoctonia on wheat in India, ascribed by Subramaniam (9) to *Rhizoctonia destruens* Tassi, which was considered to be the same fungus as *Sclerotium rolfsii* Sacc. Godfrey (3) also reported the latter fungus on wheat in the United States. Shaw and Ajrekar (8) have also reported *Rhizoctonia napi* West on wheat and oats in India. Peyronel (4) isolated a species of Rhizoctonia, which he considered was possibly a simple variation of *R. solani*, from the base of the culm and from roots of wheat affected by root rot in Italy. Fairly definite statements have also been made of Rhizoctonia causing root rot of wheat by Bessey[1] and by Richards[2] in the United States, but in neither case was a description, either of the disease or of the fungus, given.

In a preliminary note Samuel (7) reported a Rhizoctonia as causing a definite seedling disease in wheat and oat fields in South Australia, and the object of the present paper is to give a fuller description of this disease.

THE DISEASE

The disease affects wheat, oats, barley, and pasture plants on what is known as the "mallee" soils of southern Australia (Fig. 1). These soils take their name from the native vegetation, which was a dwarf Eucalyptus scrub known as mallee. They are usually characterized by alternating sand ridges and red-loam flats overlying a shallow limestone. They are decidedly alkaline, mostly having a reaction between pH 8.0 and pH 9.0.

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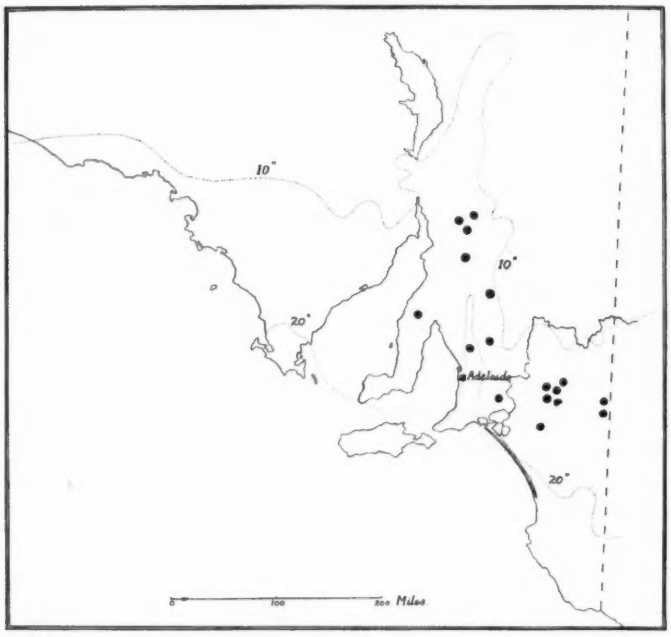

FIG. 1. Map of the southern portion of South Australia, showing localities from which the seedling disease of wheat and oats, caused by *Rhizoctonia solani*, has been reported. The area enclosed by the 10-in. and 20-in. rainfall isohyets roughly delimits the mallee soils.

The average annual rainfall is from 12 to 18 in., falling mainly in the winter.

The disease itself is not of great economic importance. It occurs in patches that vary from 2 ft. or less in diameter to large irregular areas an acre or more in extent. Small patches some 3 to 6 ft. across are perhaps the commonest. Usually only an odd patch or two can be found in a crop, but occasionally the infected spots are more numerous and coalesce into larger areas. The margins of the spots are usually sharply defined; they become very noticeable as the crop grows up, owing to the hollow space left, with small stunted seedlings that usually die later, the space becoming filled with weeds by harvest time. In a number of cases the patches have persisted in the same spots in the field over several years and have even enlarged somewhat, in spite of a year's fallow.

Symptoms. The diseased plants are noticeable at a very early stage, frequently before the crop has begun to tiller. They appear spindly and stunted, with stiff, rolled leaves pointing upwards (Fig. 2, A). In the case of oats there is often considerable purpling of leaves and stems, especially later, when dry weather sets in. The affected plants do not tiller and

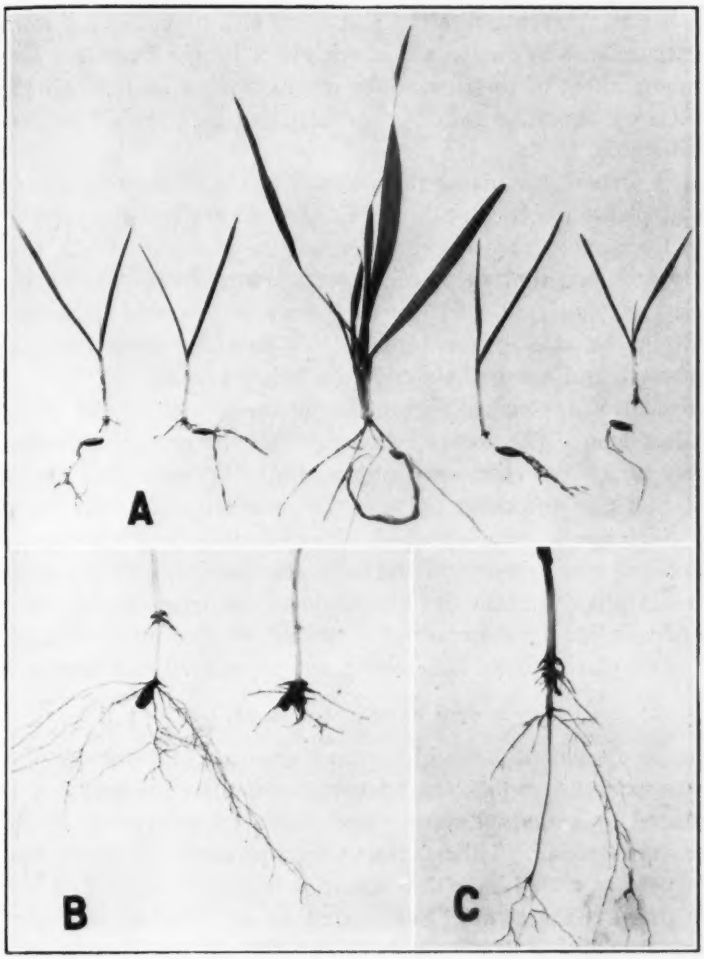

FIG. 2. A. Oat seedlings, showing the characteristic stiff and stunted appearance due to the attack of the roots by *Rhizoctonia solani*. Healthy plant in the center. B and C. Wheat and oat roots, respectively, showing killing back of both seminal and nodal roots by *R. solani*.

usually remain practically at a standstill during the winter months of June, July, and August. Then, as the weather becomes warmer, all the plants in many of the patches die without having attained a height of more than about 6 in. In a few cases, when the attack is apparently not so severe, some of the plants are able to form fresh roots with the advent of the warmer weather and may make a fairly good recovery. This may happen with isolated plants in the patches or occasionally with all the plants in a patch. In the latter case the patch is noticeable when the crop is ripening

in November as an area where the plants are still green, owing to the delay in maturity caused by the initial check given by the fungus. By far the most common effect of the disease, however, is the killing of all the plants when still very small, so that by harvest time the "hole" in the crop is filled with weeds.

It is the roots of the plants that show the most characteristic symptoms. If diseased plants are dug up in the earliest stages and their roots washed out, it is found that the tips or intermediate portions of the root are so vigorously attacked that the tissue loses all turgidity. The effect is very reminiscent of "damping-off" on the stems of crowded herbaceous seedlings, but in the case of the cereals it is only the roots that have been found affected, and never the stem, even below ground.

The flaccid, water-soaked appearance of the tissues in this earliest stage does not last long. The cortex rots away and the central cylinder breaks, so that the affected part is soon nothing but a brown stub (Fig. 2). The killing of root tips and other parts of the roots stimulates the formation of laterals, which are in turn attacked. The result is a rather characteristic, much-branched root system in diseased plants, mainly in the seminal roots, for the roots put out from the first node are usually quickly killed back. This, in fact, is largely the reason for the severe effect of the fungus on the plants, for they are unable to establish any secondary root system.

THE CAUSAL ORGANISM

When the flaccid portions of the root seen in the earliest stages of the disease are examined under the microscope the cortical tissues are seen to be penetrated by abundant stout, vigorous, colorless hyphae full of a clear refringent protoplasm. This stage is very transitory, however, and by the time the root has rotted back to a stump it is almost impossible to find any active-looking hyphae at all. Corresponding with this, it is easy to isolate the fungus during the first stage but very difficult to obtain it at the later stage, when saprophytic fungi and bacteria are already established on the browned stub of the root. At this later stage occasional long, thin, dark brown hyphae with typical Rhizoctonia branching are often seen in the soil outside the root.

The vigorous intracellular hyphae average 10 μ in diameter, but when passing directly across cortical cells they may attain a diameter of 17 μ, mainly because they are somewhat swollen just preceding the constriction for penetrating the cell walls (Fig. 3). At the height of their activity these hyphae may be so packed inside the cortical cells that they seem almost to have replaced the entire cortex. With the disintegration of the tissue the hyphae rapidly become vacuolate, and when the root has been reduced to a stub the long, thin, brown, distributive hyphae 7 μ in diameter

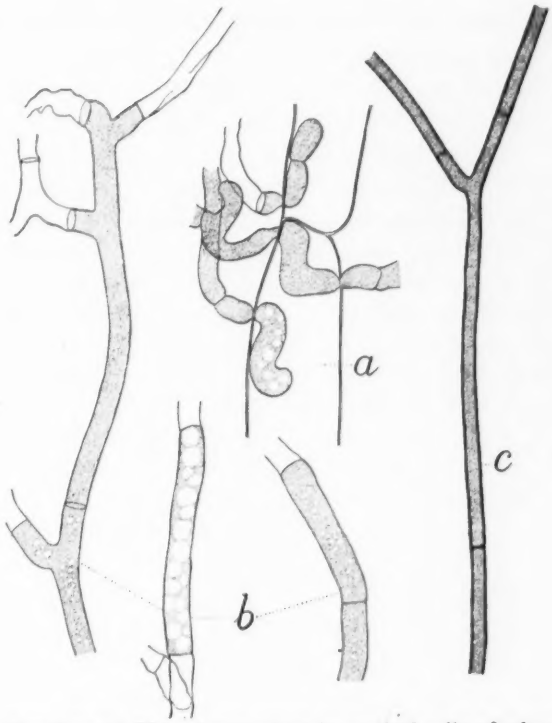

FIG. 3. A. Mycelium of *Rhizoctonia solani* in cortical cells of wheat root. Hyphae colorless and full of clear, refringent protoplasm. B. Stages in rapid vacuolation and emptying of these hyphae. C. Thin, brown "distributive" hyphae found in the soil surrounding roots after disintegration of the cortical tissues.

are the only ones to be seen. These are the hyphae that evidently grow through the soil and cause fresh infections when they come in contact with healthy roots. The origin of the brown distributive hyphae from the colorless, actively parasitic hyphae and, *vice versa,* have both been seen.

Isolations have been made from diseased patches in widely scattered localities, and in all cases a fungus has been obtained that on potato-dextrose agar gives a vigorous light brown mycelium of characteristic Rhizoctonia character, which later forms rather dark brown sclerotia of irregular size and shape. This fungus has been compared with 3 strains of *Rhizoctonia solani* isolated from potatoes and, as shown in the following paragraphs, is practically indistinguishable from these strains. Of the 3 strains of *Rhizoctonia solani* used, 2 were kindly supplied by Dr. W. L. Waterhouse, 1 having been isolated from New South Wales potatoes and the other received by him from Minnesota, U. S. A. The 3rd strain was freshly isolated from sclerotia on South Australian potatoes.

COMPARISON WITH RHIZOCTONIA SOLANI FROM POTATOES

(1) *Growth on potato-dextrose agar at different temperatures.* The 2 isolates compared in this test were No. 161, Rhizoctonia from wheat, Tarcowie, South Australia, and No. 171, *Rhizoctonia solani* from potatoes, New South Wales. The experiment was carried out in a multiple temperature incubator with 14 compartments. Each strain was grown in triplicate at each temperature; the inoculum was from mycelium from colonies previously grown at the same temperature; the agar was poured deep; and all other necessary precautions were taken for accurate comparison. Daily measurements of colony diameter were made, and figure 4 shows the curves obtained for growth at different temperatures by plotting the measurements at the end of 5 days.

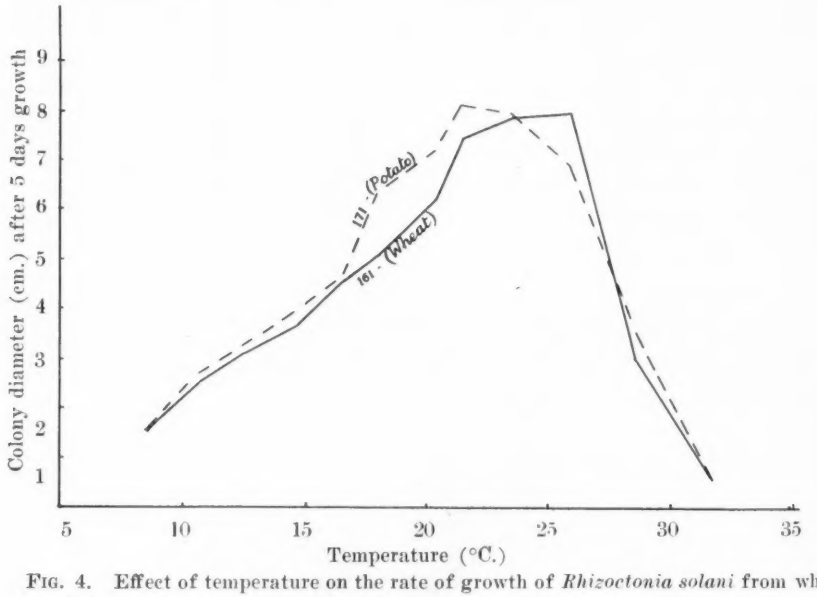

FIG. 4. Effect of temperature on the rate of growth of *Rhizoctonia solani* from wheat roots and from potatoes on potato-dextrose agar.

The cardinal temperatures for both fungi may be given as: maximum, 32° C.; optimum, 23–26° C.; minimum, 4° C. However, there was very little growth above 28° C., and below 13° C. the growth became extremely thin and spidery, scarcely visible to the naked eye.

(2) *Growth on potato-dextrose agar adjusted to different reactions.* The methods used in this experiment followed very closely those used by Webb and Fellows (11). Six isolates of the cereal Rhizoctonia and 2 isolates of *Rhizoctonia solani* from potatoes were compared. Measurements of colony diameter were made at the end of 3 days in order that the progressive

change towards acidity of the more alkaline agars should not affect the results too much.

The optimum reaction for the growth of the cereal Rhizoctonias lay between pH 6.0 and 6.5, while the optimum for the 2 isolates of *Rhizoctonia solani* from potatoes was perhaps slightly more alkaline, pH 6.5 to 7.0. However, the influence of reaction upon the rate of growth of the isolates from both hosts showed a good general correspondence over the whole range (Fig. 5). The separation of the curves in the vertical scale of this figure is a measure of the vigor of growth of different isolations only; the freshly isolated culture No. 183 from South Australian potatoes showed a very vigorous growth.

(3) *Cross-inoculation tests.* The cultures used were No. 161, Rhizoctonia from wheat, Tarcowie, South Australia, and No. 171, *Rhizoctonia*

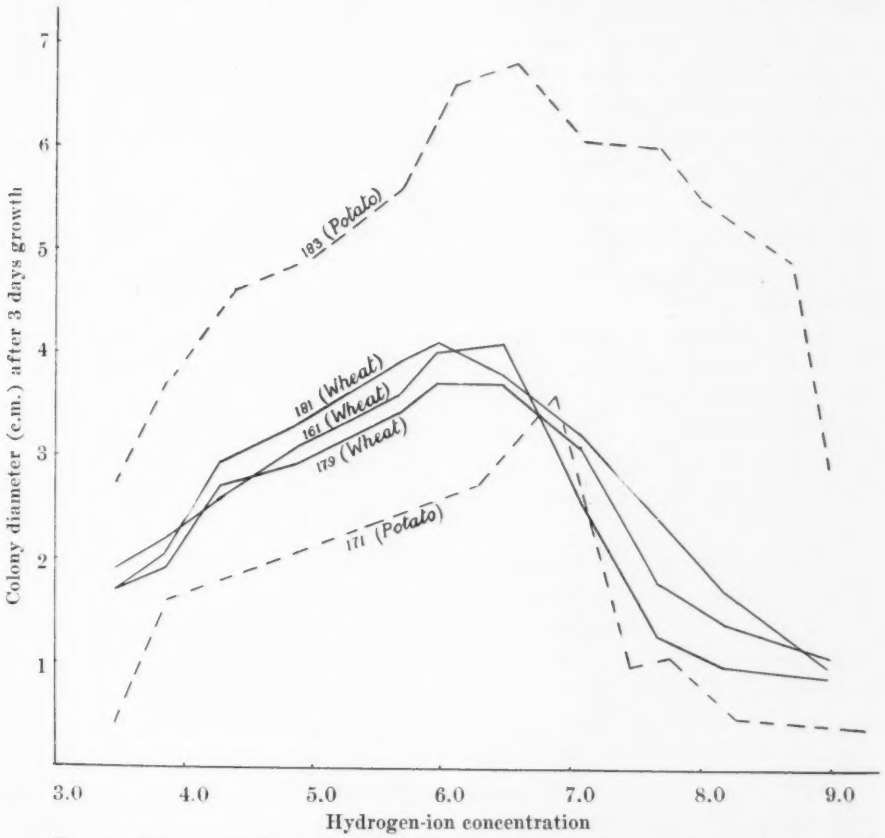

FIG. 5. Effect of hydrogen-ion concentration of the medium (potato-dextrose agar) on the rate of growth of 3 isolates of *Rhizoctonia solani* from wheat roots and 2 from potatoes.

solani from potatoes, New South Wales. The inoculum was multiplied on a mixture of sterilized oat and barley kernels, and lots of 10 gm. or 60 gm. of inoculum were used per 6-in. pot. Each fungus was tested on potatoes, wheat, oats, and barley, both in pure sand and in a sand-soil mixture.

After only 3 weeks' growth the inoculated pots were noticeably behind the controls. On washing out, the roots in all inoculated pots were found to be attacked, including cereals inoculated with the potato fungus and potatoes inoculated with the cereal fungus. The affected portions of the roots were browned, flaccid, and easily broken. Microscopical examination showed that the cortex was densely penetrated by the characteristic vigorous, refringent Rhizoctonia mycelium. Comparison of the severity of attack in the various pots showed that the cereal Rhizoctonia had affected the cereals somewhat more severely and that the potato Rhizoctonia had affected the potatoes more severely, as judged both from the number of points of attack on the roots and from the amount of mycelium in the cortex. The experiment demonstrated beyond question, however, that the Rhizoctonia from wheat would attack potatoes and that *Rhizoctonia solani* from potatoes would attack cereals. As mentioned above, this last point had been previously demonstrated by Rayllo (5).

(4) *Effect of soil temperature on pathogenicity.* Two experiments have been done in soil temperature tanks of the Wisconsin type and both showed that the cereal Rhizoctonia is a more vigorous parasite of wheat at low temperatures.

As an example of these experiments, Rhizoctonia No. 161 from wheat, Tarcowie, South Australia, was multiplied on oat-barley-kernel medium and 100 gm. of inoculum per 8-in. container was used in a soil-sand mixture, maintained at 50 per cent saturation by frequent weighings and addition of water at the correct temperature. Federation wheat was planted in the containers and the tanks were maintained at temperatures of 12, 17, 22, 27, and 32°.

The containers were washed out for examination of the roots 3 weeks after planting. The plants were most severely infected in the soil maintained at 12° C.; the majority of the roots were little more than browned stubs and the tops of the plants were correspondingly stunted. The plants were still fairly severely attacked at 17° C., but at 22° there was no perceptible effect on the tops and a much less severe root infection; at the higher temperatures the effect was very slight. Microscopic examination of the roots showed a relatively small amount of infection at the higher temperatures, and what mycelium there was in the cortex had none of the vigorous appearance characteristic of the infections at 12 and 17° C.

DISCUSSION

The isolations of Rhizoctonia from diseased patches in wheat and oat crops have agreed so closely in all characters tested with isolations of *Rhizoctonia solani* from potatoes that the cereal fungus may be definitely referred to this species.

In field inspections the very characteristic stunting effect of the fungus in the early seedling stage is liable to confusion with only one other disease in South Australia, namely, the nematode disease caused by *Heterodera schachtii* (1). The two diseases can be immediately differentiated by examining the roots, however. The nematode causes small but definite root-knots, with tufts of lateral roots arising from them, whereas plants affected by Rhizoctonia have the roots killed back to brown stumps.

The occurrence of the disease in the field during the seedling stage in winter corresponds with what one would expect from the soil-temperature-tank studies. The fungus is most active as a parasite at low temperatures, and seedlings that are not killed before spring usually recover in the warm weather. Generally similar temperature relations were obtained by Richards (6) in his study of the effect of *Rhizoctonia solani* on potatoes, and a number of other workers have also reported more vigorous parasitism on various plants at low to medium temperatures. However, definitely higher optimum temperatures for disease production have been reported by Walker (10) for this fungus on cotton and by Dickinson (2) for brown patch of turf.

It is curious that the fungus seems restricted entirely to the roots and is unable to attack the stems, even underground. This is in contrast to its effect on potatoes, cotton, and in brown patch of turf, and other diseases.

No sclerotia have been found in the field, although they were occasionally found on inoculated plants in pure sand. The fungus probably oversummers in the form of the thin, dark brown, distributive mycelium.

The fungus is scarcely of sufficient economic importance yet to call for definite experiments on control. Since the patches are still visible when the land is left out to grazing, owing to the disease affecting the pasture grasses and other plants, the leaving of the land to pasture is of no avail. The low rainfall in areas where the disease occurs practically prevents any crop other than cereals being grown in the rotation, and all the cereals are susceptible. Fallowing is the most hopeful method of control, but there have been a number of instances in which 1 year's fallow has not rid the soil of the fungus. The last 5 or 6 years in South Australia have been very dry, however, and it is possible that good working of the land in a year of normal rainfall will prove effective.

SUMMARY

Rhizoctonia solani is shown to be responsible for a definite seedling disease of wheat, oats, and barley on the low-rainfall, alkaline, "mallee" soils of South Australia.

The disease occurs usually in small, definite patches. The fungus attacks the roots, causing them to die back to brown stumps, and the affected seedlings remain stunted and later die. Pasture plants also are affected.

The disease is most active at low soil temperatures (12–18° C.).

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TRICHODERMA LIGNORUM AS A PARASITE OF OTHER SOIL FUNGI[1]

R. WEINDLING

(Accepted for publication February 23, 1932)

The following observations regarding the parasitism of *Trichoderma lignorum* (Tode) Harz were made in connection with a study of the damping off of Citrus seedlings.[2] A fast-growing strain of *Rhizoctonia solani* Kühn[3] was most frequently isolated from damped-off seedlings. In inoculation experiments it proved extremely virulent, especially on the recently germinated seedlings of sour orange, sweet orange, and grapefruit. *Phytophthora parasitica* Dastur and *Pythium* spp. also were isolated, but much less frequently than the first fungus. Moreover, these last two fungi appeared to be virulent only under certain favorable environmental conditions. Secondary invaders, repeatedly isolated from diseased roots, were *Fusarium* spp. and a strain of *Trichoderma lignorum*. By means of numerous inoculations both organisms were shown to be nonpathogenic to Citrus seedlings.

During these experiments Trichoderma hyphae were observed coiling around Rhizoctonia hyphae and destroying colonies of this fungus. The same result was obtained repeatedly on several culture media, the Petri dishes being inoculated simultaneously with pure cultures of both fungi.

In studying the peculiar parasitism of this fungus the following modes of attack were observed. Side branches of the advancing Trichoderma mycelium coil more or less tightly around aerial hyphae of Rhizoctonia or grow along them in straight or wavy lines. The protoplasm of the attacked hyphae coagulates and the cells lose their vacuolate structure (Fig. 1, A–D). Often the host hyphae break at a septum and some of the granular contents exude. Such a location seems to be particularly attractive for Trichoderma hyphae, which increase rapidly in number and diameter (Fig. 1, E, F; Fig. 2, A). Growth of the host hyphae stops almost abruptly with the initiation of the attack by the parasite. The more advanced the stage

[1] Paper No. 266, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] The investigation was made at the University of California, first, under the direction of J. T. Barrett, at Berkeley, later under the direction of H. S. Fawcett, at Riverside, who had originally suggested the damping-off problem. Valuable suggestions and help were also obtained from L. J. Klotz, T. E. Rawlins, and several other staff members of the Division of Plant Pathology at Berkeley and at Riverside.

[3] For identification of the organisms, thanks are due to C. L. Shear, of Washington, D. C., and S. F. Ashby, of the Imperial Mycological Institute, Kew, England.

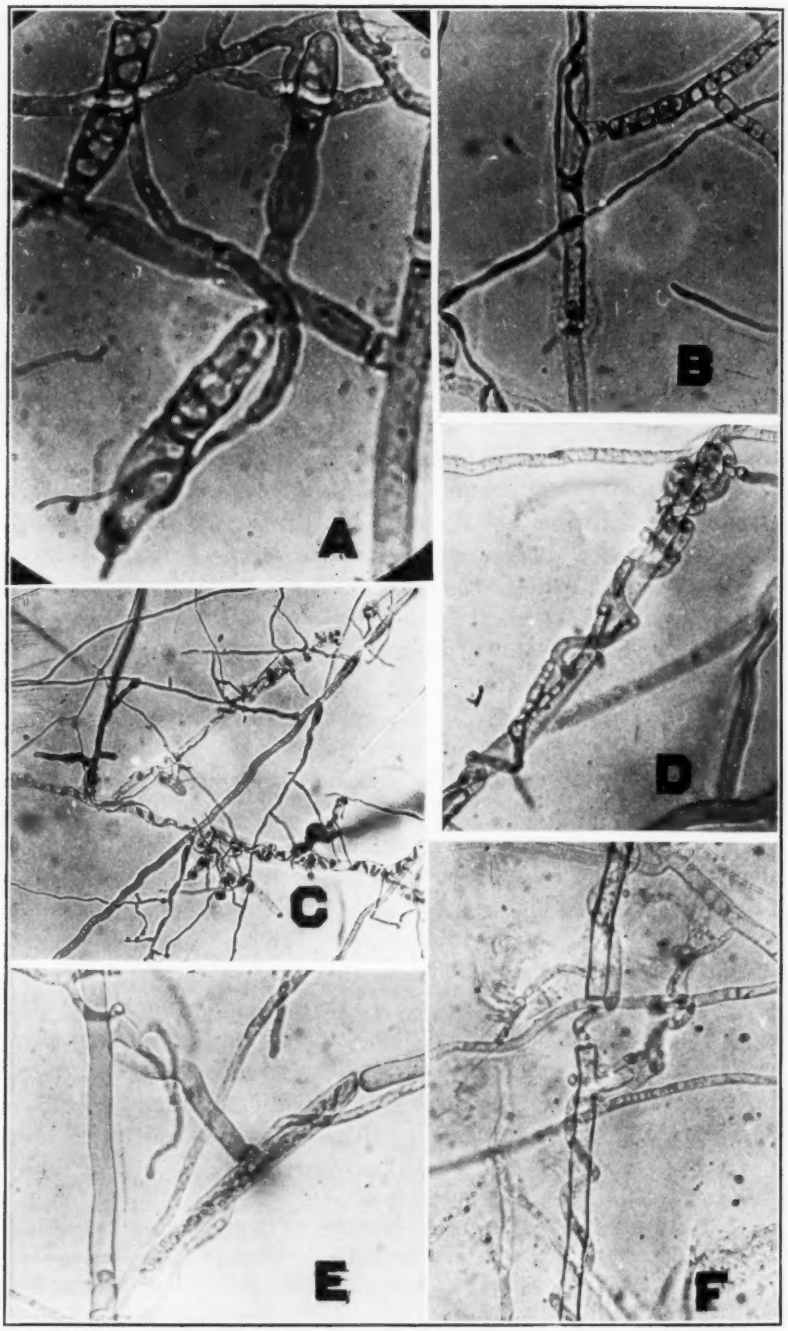

FIG. 1. A and B. Beginning of aerial attack on Rhizoctonia (citrus strain) by Trichoderma. Note stages in disappearance of vacuolate structure. The hyphae of smaller diameter are Trichoderma. A, × 660; B, × 330. C and D. Trichoderma coiling around host hyphae. C, × 110; D, × 330. E and F. Rhizoctonia hyphae breaking up at septa as the result of attack. × 330.

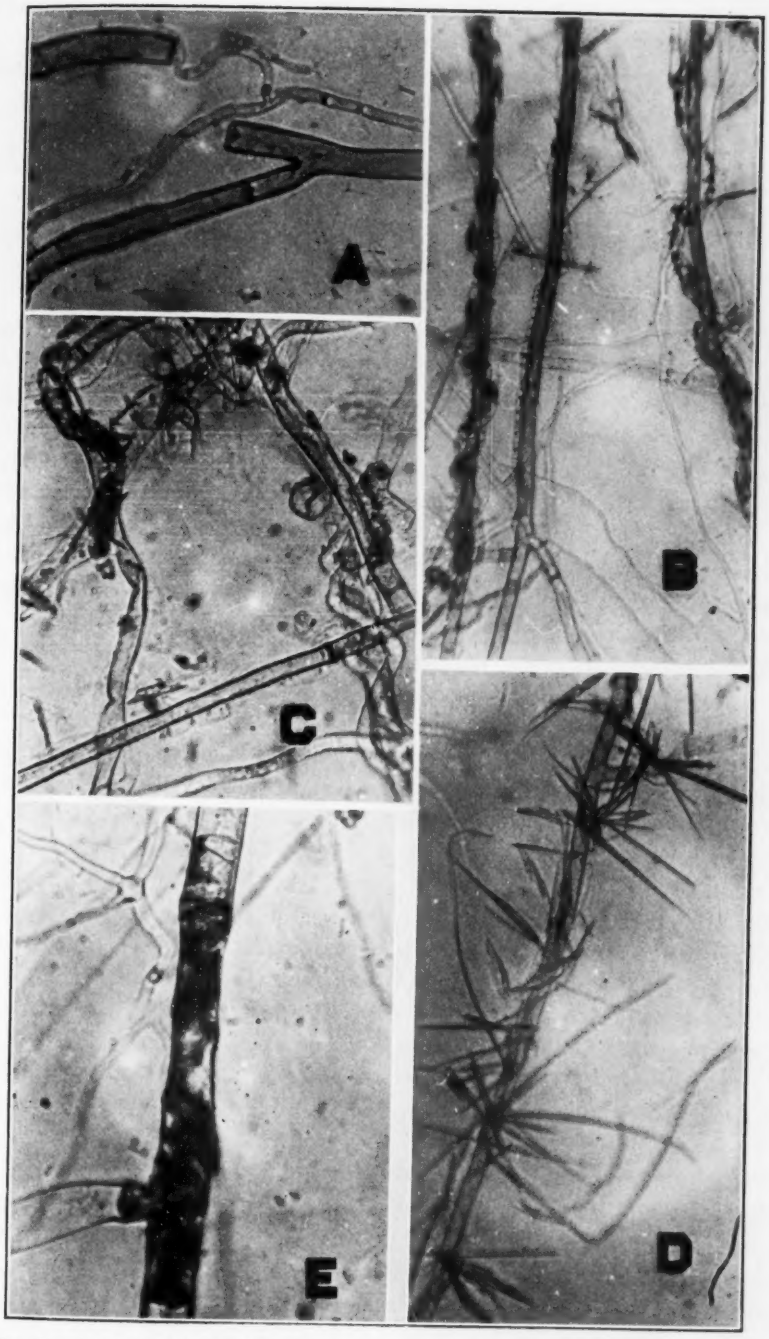

FIG. 2. A. Rhizoctonia (citrus strain) cells breaking up at septa as the result of aerial attack by Trichoderma. B to D. Autolysis stages of some of the attacking Trichoderma hyphae, showing the yellow protuberances that break up later into crystals. B, × 190; C and D, × 330. E, Rhizoctonia growing on the surface of medium, with Trichoderma close but not in contact. Host hypha becoming filled with dark yellow material. × 800.

of attack, the paler the host mycelium becomes and the less readily it stains with haematoxylin and other dyes. Frequently, coiling branches of the parasite form protuberances containing light-yellow granules. These protuberances finally break up, releasing numerous sheaves of yellow, needle-like crystals (Fig. 2, B–D). Most of the attacking hyphae, however, remain alive, and almost none of the thicker, nonattacking Trichoderma threads disintegrate. Also, living Rhizoctonia cells are occasionally found after all surrounding hyphae of this fungus seem to be killed.

While, with the "aerial attack" just described, the effect of the parasite on the host hyphae seems to be rather localized, this is much less the case below the surface of the medium. In the medium coiling occurs rarely, but, as the Trichoderma colony advances, host hyphae die and may break up in regions in advance of the invading parasite (Fig. 3, A). Dark-yellowish masses accumulate in parts of some of the attacked hyphae, which later break up, forming masses of dark yellowish, star-like crystals (Fig. 3, B–E). The arrangement of these crystals frequently suggests explosive-like violence during the disintegration of the host hyphae. Colorless media appear aniline yellow (9) wherever the yellow pigment of hyphae and crystals is formed in large quantities. Occasionally, one may find a few Trichoderma segments becoming dark yellow and breaking up in the same way as the Rhizoctonia.

Also, in very old pure cultures of Trichoderma, sheaf-like, aerial crystals, as well as star-like, submerged ones, have been found, but always much less frequently than in the mixed cultures. In pure Rhizoctonia cultures, when killed by chloroform vapor, the hyphae lose their vacuolate structure in a manner similar to that shown during the first stage in Trichoderma attack. But they neither break at septa nor do they form yellow crystals.

While external parasitic attack is prevalent, internal parasitism seemed to occur in some hyphae of a strain of *Rhizoctonia solani* isolated by H. S. Fawcett from potatoes (Fig. 3, F, G). The latter fungus does not form true anastomoses with the Rhizoctonia strain from Citrus. Moreover, the strain from potato is essentially nonparasitic on Citrus seedlings and appears to be more susceptible to the attack of Trichoderma, especially in water-agar media.

With *Pythium* spp. and *Phytophthora* spp., grown in conjunction with Trichoderma, similar star-like and sheaf-like crystals were formed from disintegrating hyphae (Fig. 4, A). In general, these fungi seem to be more readily overcome by the parasite than does the Rhizoctonia. Other fungi found up to now that are more or less susceptible to the attack of Trichoderma are: *Sclerotium rolfsii* Sacc. (Fig. 4, B) and *Rhizopus* spp. (Fig. 4, C). Several variations in the mode of parasitism have been observed on

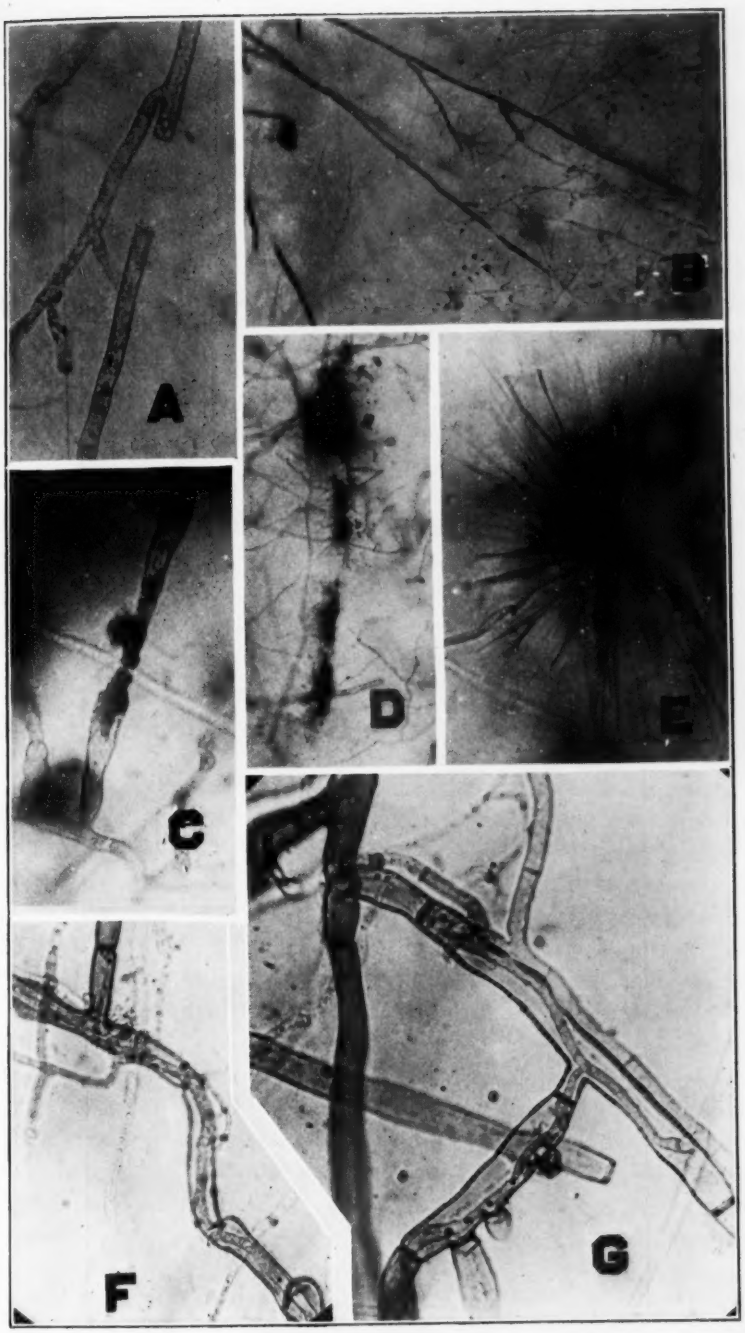

FIG. 3. A–E. Submerged attack of Rhizoctonia (citrus strain) by Trichoderma. A. Dead Rhizoctonia hypha breaking up at a septum and releasing granular contents. × 300. B–E. Stages in the disintegration of host hyphae. B, × 65; C, × 330; D, × 160; E, × 450. F and G. Internal parasitism of Trichoderma in *Rhizoctonia solani* (strain from potato). Note connections of coiling hypha with internal hypha in figure F. F, × 400; G, × 600.

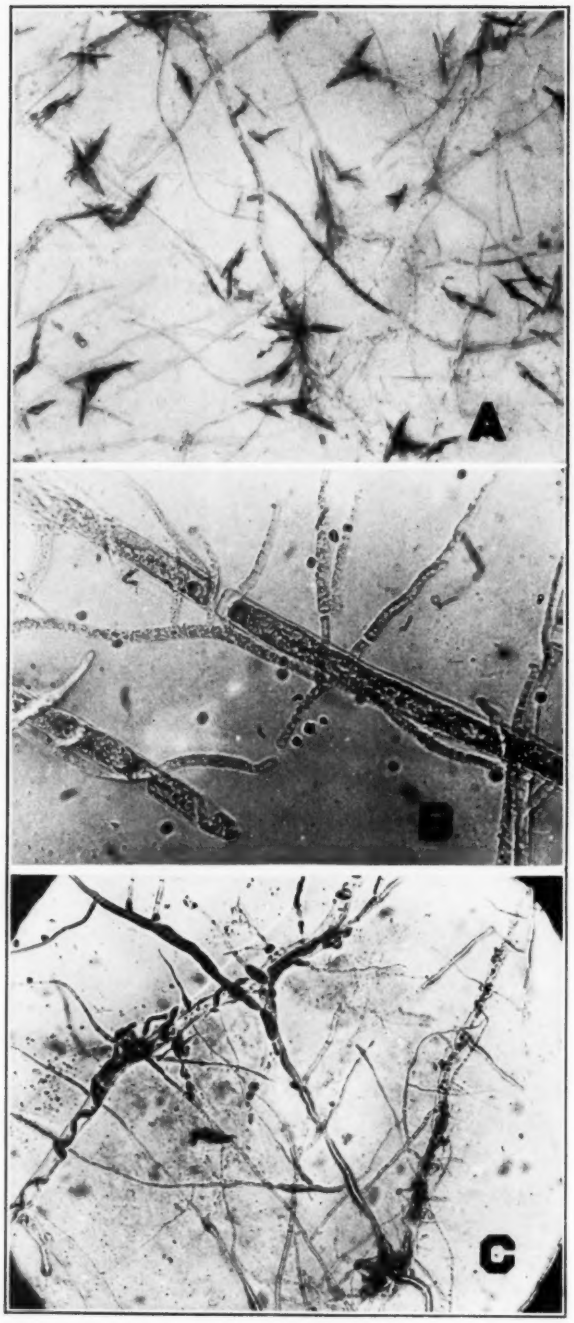

FIG. 4. A. Stages in the disintegration of Pythium hyphae when attacked by Trichoderma. Note sheaves of crystals on the surface of the culture medium. × 80. B. Initial attack of Trichoderma on *Sclerotium rolfsii* showing one break at a septum. × 660. C. Coiling of Trichoderma around Rhizopus hyphae. × 110.

hosts other than Rhizoctonia and on this fungus when grown on different media or attacked in later stages of growth. It is beyond the scope of this preliminary paper to describe these modifications. Essentially, the manner of the Trichoderma attack seems to be similar to that outlined.

Most of the material for the pictures was obtained by removing small areas of glucose-agar plate cultures to glass slides. The cultures had been incubated at 21 or 28° C. in a moist atmosphere. Slight drying-out of the media stops the growth of Trichoderma. Some material was grown on agar films on cover slips that were sealed to glass slides; some of the material was not stained, and some stained with vital stains (Congo red 1:80,000).

It is conceivable that in some cases Trichoderma may act merely as a competitor for food, while incidentally killing other fungi in mixed cultures. The aerial attack, however, suggests strongly that substances from the host hyphae are utilized as nutrients. Moreover, while the fructification of the parasitic fungus is usually delayed where opposed colonies meet, the mycelium as well as the fructification of Trichoderma seems eventually to be more abundant in mixed than in pure cultures.

That parasitism, independent of any medium, may take place whenever the parasitic fungus is actively growing and has sufficient moisture, was demonstrated by the following procedure. Rhizoctonia hyphae were permitted to grow out of pure cultures onto sterile slides that did not touch the medium in the Petri dish. Similarly, Trichoderma hyphae were obtained on cover slips free from media. The inverted cover slips were then sealed to the slides, leaving a small air space between them and allowing the entrance of moist air through openings in the sealing. On these slides, which were kept in moist Petri dishes, branch hyphae of Trichoderma growing along the host filaments were observed to acquire a much larger diameter in an equal period than the main hyphae from which they originated.

The writer has not yet determined whether enzymes or toxins are the active principles in this parasitic action. Apparently, to be effective in aerial attack, these substances must be brought into close contact with the host hyphae, since there is no medium immediately present for diffusion of these products. This localization of attack seems to bring about destruction of both the fungus host and parts of the parasite, itself, the attacking hyphae of the latter eventually being autolyzed. When the attack is within the medium, however, the destructive products appear to diffuse ahead, killing the host hyphae and having little autolytic effect on the parasite. On the surface of the medium, there is a gradation of these two distinct modes of attack. Hyphae filled with yellow material have been observed at that location, probably giving rise to aerial, sheaf-like crystals (Fig. 2, E). This suggests the possibility that the sheaves of aerial crys-

tals and the submerged, star-shape crystals may be chemically the same, their difference in physical appearance being effected by the surrounding medium. In the chemical tests that follow, the medium may also have had some influence on the results. The aerial crystals were found to be soluble in chloroform. The submerged yellow hyphae and crystals gave a deep red color with 20 per cent KOH. This reaction may be due to the presence of a pigment similar to that described for certain Penicillia and Aspergilli (2).

Antagonistic effects among microorganisms have been frequently observed, and the literature on this subject has been ably reviewed elsewhere (4). Parasitism among fungi has been reported for many organisms (12), but, excepting in the Mucorales, it has not often been described in detail (1, 8). The perfect stage of *Trichoderma lignorum* is said to be a species of the genus Hypocrea. Other species of this genus have been reported as parasites on fructifications of many Basidiomycetes (10, 12). In the inoculation of soil supporting beet and pine seedlings, it was shown (3, 5) that parasitic and saprophytic fungi together caused less loss than parasitic fungi alone. Attempts have been made to control parasitic organisms by their antibiotic enemies (6, 7).

The research reported in this paper suggests that under certain conditions Trichoderma might be used for the biological control of fungus diseases. For example, in damping off, in which the most susceptible period of certain seedlings is relatively short, an abundant inoculation of the soil with the protective organism may tide them through the critical period, before the plant parasites have become reestablished. It will probably be difficult to keep Trichoderma dominant in the surface soil because of its tendency to self-digestion and because the vegetative form of the fungus is dependent on continuous moisture. It is reported to be a fungus *par excellence* of water-logged soils (11); that is, it is favored by conditions good for most parasitic fungi and bad for most plants. Preliminary pot experiments with Citrus seedlings have revealed certain difficulties but have shown some striking cases of the protective action of Trichoderma in preventing damping off. These data as well as others that are being collected will be reserved for later publication.

SUMMARY

In the course of an investigation of damping off of Citrus seedlings, a strain of *Trichoderma lignorum* has been found to parasitize a number of pathogenic soil fungi in cultures, *i.e.*, *Rhizoctonia solani*, *Phytophthora parasitica*, *Pythium* spp., *Rhizopus* spp., and *Sclerotium rolfsii*.

The inhibition and death of the host hyphae were brought about in two ways: (a) in aerial hyphae by close contact or by coiling of Trichoderma

around them; (b) in submerged hyphae by action at a little distance more frequently than by contact.

Trichoderma hyphae were also found to attack Rhizoctonia hyphae, when both fungi were free from any connection with nutrient media.

Based on preliminary pot experiments, the possibility is suggested of controlling certain pathogenic soil organisms by abundant inoculation of the soil with cultures of *Trichoderma lignorum*.

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REACTION OF MARTIN WHEAT TO THREE PHYSIOLOGIC FORMS OF TILLETIA TRITICI[1]

WILLIAM K. SMITH

(Accepted for publication February 8, 1932)

Within *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn, the 2 species of fungi causing stinking smut of wheat, the presence of numerous physiologic forms has now been demonstrated. Practically all determinations have been made on the basis of the percentage of bunt on differential varieties of wheat inoculated with different collections. However, in some preliminary trials with a large number of collections, Bressman[2] observed indications of differences between some collections in the texture of the smut ball, in the occurrence of masses of bunt spores on the outside of the glumes, and in the optimum temperature for spore germination. Rodenhiser[3] found a significant difference between 2 physiologic forms of *T. tritici* in the stunting of the bunted culms of Little Club.

From the collections of bunt reported by Gaines[4] in 1928, three physiologic forms of *Tilletia tritici* were later distinguished by the reaction of a number of differential varieties of wheat. As was pointed out in another paper,[5] one physiologic form, T2, could readily be recognized as distinct from T1 and T3 by the percentage of bunt on various differential varieties but particularly by the reaction of Martin (C. I. 4463), a variety of *Triticum vulgare* Vill. In a rod-row test sown in the fall of 1928, Martin was bunt-free when inoculated with T1, gave 19 per cent of bunted heads with T2, and 71 per cent with T3, while Hybrid 128 (C. I. 4512) was uniformly susceptible to all forms. In later trials, the reaction of Martin has approximated that observed in 1929. T2, however, can also be differentiated from T3 by the appearance of the bunted spikes of Martin. Because the spikes of this variety, invaded by T3, are indistinguishable from those invaded by any of the physiologic forms of *T. tritici* other than T2 or of *T. levis* in tests at the Washington Agricultural Experiment Station, it is apparent that T2 can be distinguished from any of these

[1] Published as Scientific Paper No. 218, College of Agriculture and Experiment Station, State College of Washington.

[2] Bressman, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oregon Agr. Exp. Sta. Bul. 281. 1931.

[3] Rodenhiser, H. A. Stunting of wheat caused by *Tilletia levis* and *T. tritici*. Jour. Agr. Res. 43: 465–468. 1931.

[4] Gaines, E. F. New physiologic forms of *Tilletia levis* and *T. tritici*. Phytopath. 18: 579–588. 1928.

[5] Smith, W. K. Inheritance of reaction of wheat to physiologic forms of *Tilletia levis* and *T. tritici*. Jour. Agr. Res. In press.

forms by the reaction of Martin. Barrus[6] has called attention to the significant differences between healthy and bunted culms in a white variety of *T. vulgare* similar to Dawson's Golden Chaff, and approximately the same differences are apparent in Martin between bunt-free culms and culms invaded by T3; it is not necessary, therefore, to enumerate these differences. A comparison will, however, be made between culms of Martin smutted with T2 and culms smutted with T3. Material adequate for this comparison was grown from seeds planted in adjacent rows in the fall of 1930, each row being inoculated with one of the three physiologic forms.

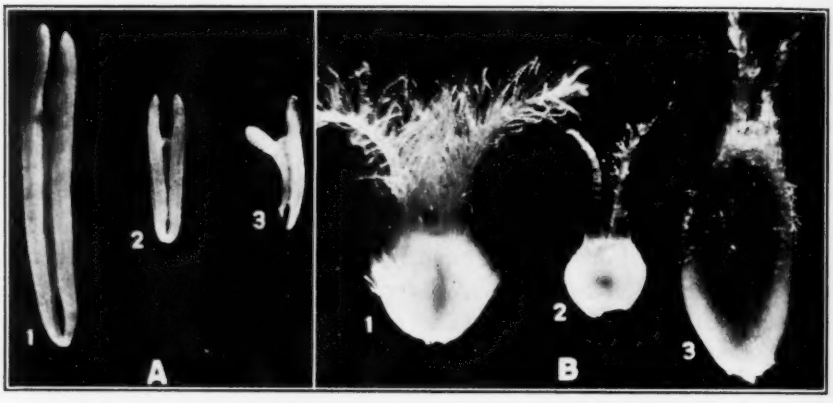

FIG. 1. A. Anthers of Martin wheat: 1, from bunt-free flower; 2, from flower infected with physiologic form T2; 3, from flower infected with T3. B. Ovaries of Martin wheat: 1, bunt-free; 2, infected with T2; 3, infected with T3.

The first observations were made on diseased culms when the spike had partly emerged from the uppermost leaf sheath. At this stage of growth the following characteristics were noted: (1) On T2 culms, *i.e.*, culms of which the spike was invaded by physiologic form T2, the length (4 to 12 cm.) and width (3 to 6 mm.) of the uppermost leaf were considerably less than the length (15 to 24 cm.) and width (10 to 15 mm.) of the corresponding leaf on healthy and on T3 culms; moreover, the T2 leaf was markedly twisted. (2) The length of the anthers (2.2 to 3.2 mm.) in the flowers of the T2 spike was somewhat greater than that in the flowers of the T3 spike (1.3 to 1.9 mm.); the anthers in the T2 flowers were less distorted and were greener than those in the T3 flowers (Fig. 1, A). (3) The length of the ovary (1 to 1.8 mm.) on T2 spikes was on the average slightly less than that in bunt-free spikes, while the ovary in each was approximately of the

[6] Barrus, M. F. Observations on the pathological morphology of stinking smut of wheat. Phytopath. 6: 21–28. 1916.

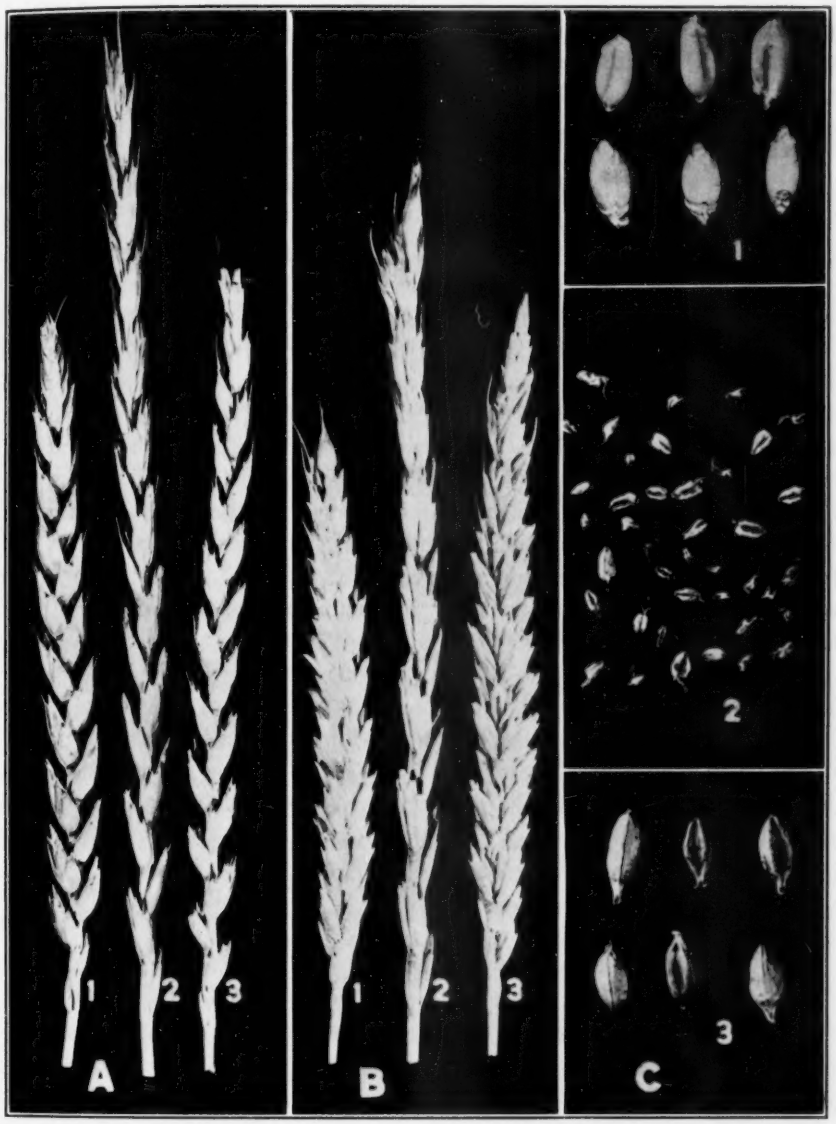

FIG. 2. A and B. Mature spikes of Martin wheat: 1, bunt-free; 2, infected with T2; 3, infected with T3. C. 1, bunt-free kernels; 2, bunt balls from infection by T2; 3, bunt balls from infection by T3.

same color—white to pale green (Fig. 1, B); these contrasted sharply with the dark-green and swollen ovary (2.2 to 3.7 mm. in length) of the T3 spikes. The stigmas in the T2 and T3 heads were poorly developed in comparison with those in bunt-free plants.

At maturity the following differences are apparent: (1) Spikes infected with T2 (Fig. 2, A and B) are more lax than those infected with T3; from measurements made on heads having a comparable number of spikelets per head, the average length of 10 internodes in the middle of the head for the T2 group was $6.93 \pm .08$ cm., while that for the T3 group was $5.72 \pm .04$ cm., giving a significant difference of $1.21 \pm .09$ cm. (2) The T2 spikes are much narrower than the normally bunted spikes in which the smut balls protrude from the glumes; the T2 spikes resemble sterile spikes in appearance. This condition is due to the fact that the smut balls arising from the infection of Martin by T2, while having a considerable range in size, are very small (0.5 to 3.5 mm. in length) in comparison with smut balls arising from infection by T3 (4.5 to 6.5 mm.); the T2 smut balls are shrivelled and angular (Fig. 2, C). The spores in the abnormal smut balls seem to be in various stages of development; in some balls, particularly the larger ones, a proportion of spherical, thick-wall spores with well-defined reticulations has been observed, but there is present also a large proportion of thin-wall, apparently immature spores that are often much distorted in shape.

The reaction of Martin to T2 is a type of resistance; yet it is a resistance that is of practical value only in reducing the amount of inoculum, because, although ordinary smut balls do not develop, healthy kernels are not produced. The condition, however, is of interest in being a character by which this physiologic form may be distinguished from other physiologic forms of bunt.

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LEAF TEMPERATURE OF LETTUCE AND ITS RELATION TO TIPBURN

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(Accepted for publication February 23, 1932)

The symptoms of tipburn of lettuce were first described in 1891 by Jones (1), but he did not mention causal agency. Since that time many explanations of the cause of this disease have been offered. Although organisms have been found in tipburn tissue, there is no evidence that they cause the disease. Superabundant soil moisture and excessive application of fertilizers have been considered responsible for tipburn, and there is also a general impression that the injury is due to high temperature and rapid transpiration.

The relation of leaf temperature during the process of tipburning of lettuce has received very little attention. In order to obtain some information on this question, investigations were undertaken to study the temperature of (1) leaves during day and night, (2) various portions of leaves under identical conditions, and (3) leaves in direct and diffuse light.

A brief report of the results of these investigations was made in 1927 (2).

METHODS OF EXPERIMENTATION

The leaf temperatures in these experiments were determined by means of thermocouples pressed against the surface of the leaf, as suggested by Shreve (4). A Leeds and Northrup No. 2500-A galvanometer was used to make the determinations. In all experiments the cold junction was kept in a thermos bottle containing distilled water. The other junction was clamped upon the surface of the leaf, care being taken to make certain of a good contact. Air temperatures were always determined by a standardized thermometer, graduated to tenths of a degree and shaded from the sun but near the leaf under investigation.

The temperature determinations were made on lettuce plants grown in a greenhouse. Certain leaves were selected for a given series of determinations, care always being taken to select leaves of about the same age and with approximately the same exposure to the incident rays of the sun. In a number of instances temperature determinations were made of leaves that were in the process of tipburning.

[1] Formerly Assistant Plant Pathologist, Colorado Agricultural Experiment Station. The data reported herein were procured while the writer was a member of its staff.

[2] The writer gratefully acknowledges the helpful criticism of Drs. L. W. Durrell and E. C. Stakman in the preparation of this manuscript.

EXPERIMENTAL DATA

The temperature of an intact leaf or a plant exposed to atmospheric conditions is influenced by many factors, the most important of which are air temperature, air currents, intensity of light to which the plant is exposed, and relative atmospheric humidity. In these data only the relation of leaf temperature to air temperature has been considered in regard to tipburn of lettuce.

Temperature of different portions of the same leaf. Some determinations were made to compare the temperature of base and tip of leaves in both sun and diffuse light, the results of which are shown in table 1. The

TABLE 1.—*Average leaf temperatures of upper and lower surfaces of healthy lettuce leaves in direct and diffuse light*

Number of determinations	Average temperature				
	Air	Base of leaf		Tip of leaf	
		Upper surface	Lower surface	Upper surface	Lower surface
	°C.	°C.	°C.	°C.	°C.
		In direct sunlight			
34	22.6	20.6	20.5	21.3	21.2
		In diffuse light			
34	19.0	20.6	20.3	21.3	21.1

conditions necessary to produce diffuse light were obtained by moving plants in the shade of an 18-in. by 30-in. round galvanized can upon which the galvanometer was resting.

The data presented in table 1 show that, in the sun, the temperature of any portion of the leaves is always consistently lower than the temperature of the surrounding air. The leaf temperature in the shade, however, is just the reverse, being higher than the air temperature. For example, the average temperature of the top of the base in the sun is 2° C. lower than the air temperature, while this portion of the leaf in the shade is 1.6° C. higher than the surrounding air temperature.

Miller and Saunders (3) found that the temperature of leaves was higher than the air temperature in the sun but was cooler than the surrounding atmosphere in diffuse light. This latter statement is the reverse of the results obtained in the experiments here reported.

From the data in table 1 it is evident that the top surface of the leaf is slightly warmer than the corresponding portion on the lower surface. In the sun the top of the base of the leaves had a temperature of 20.6° C., while the lower surface was 20.5° C. Likewise, in the shade, the top of the

TABLE 2.—*Average temperature of lettuce leaves obtained under conditions favorable for tipburn*

Time of observation	Number of observations	Temperature of air	Temperature of upper surface of base	Temperature of lower surface of base	Temperature of upper surface of tip	Temperature of lower surface of tip
		°C.	°C.	°C.	°C.	°C.
4:30 p. m.	**6**	23.0	22.1	22.0	22.5	22.6
9:15 p. m.	21	23.3	22.1	21.8	21.9	22.1
12:00 m.	9	25.5	21.8	22.0	22.4	22.7
3:05 a. m.	6	28.6	23.6	24.0	22.4	23.3
4:15 a. m.	6	29.2	25.2	25.1	22.0	25.0
5:15 a. m.	6	29.3	25.0	24.9	22.1	25.3
6:10 a. m.	6	34.9	23.2	23.4	23.1	23.0
7:05 a. m.	6	31.6	23.6	23.5	24.5	24.1
8:00 a. m.	**6**	32.6	25.9	25.8	26.4	26.2

base was 20.6° C., or 0.3° C. higher than the lower surface, while the upper surface of the tip was 0.2° C. higher than the lower surface. These data correspond with those of Miller and Saunders (3), who also found that the temperature of the upper surface of the leaf is higher than that of the lower surface in both sun and diffuse light.

The temperature of the tip is higher than that of the base of the same leaf in both sun and diffuse light (table 1). In the sun the top of the tip is 0.7° C. and the lower surface 0.7° C. warmer than the respective sides of the base. Similarly, in the shade, the temperature of the upper surface of the tip is 0.7° C. and the lower surface is 0.8° C. higher than the temperature of the upper and lower surfaces of the base. These observations are similar to those of Miller and Saunders (3), who showed that the temperature of the base of leaves is always lower than that of the tip in direct sunlight.

Leaf temperatures during the night. On the night of March 8, 1926, and the morning of March 9, 1926, readings were made on lettuce plants that were in glass chambers in the greenhouse and the plants under favorable conditions for tipburn to occur. All the plants used in these experiments became tipburned between 9:15 p. m. and 3:00 a. m.

From the data in table 2 it is evident that the temperature of the leaves was considerably lower than that of the air throughout the duration of the experiment. The temperature of the tip and of the base of the leaves fluctuated considerably during this experiment, making a correlation impossible. The important point in these data is that tipburn occurred, although the leaf temperature of these plants was consistently below the air temperature.

Since some of the plants tipburned between 9:15 p. m. and 12:00 m. when the temperature of the tips was 1.2° C. and 3.7° C. below the air temperature at these hours, it appears that tipburn is not caused by excess low temperature.

DISCUSSION

On normal healthy lettuce plants in sunlight the upper surface of the base of the leaves was found to be 2.0° C. and the lower surface 2.1° C. cooler than the surrounding air temperature. In diffuse light, the upper surface of the base was 1.6° C. and the lower surface 1.3° C. warmer than the temperature of the air.

The edge of healthy leaves was found to be only 0.7° C. to 0.8° C. warmer than the base of the leaf.

Hourly temperature readings, made during the night on lettuce leaves during the process of tipburning, showed that the upper surface of the tip ranged from 0.5° C. to 11.8° C. lower than the air temperature. During

the time that tipburning was rapidly occurring (9:15 p. m. to 12:00 m.) the range was from 0.5° C. to 3.1° C. below the temperature of the surrounding air. It therefore is evident, from the data presented, that tipburn of lettuce is not caused by an excessive temperature of the leaf tissues.

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STUDIES ON BORDEAUX DEPOSITION

GEO. L. HOCKENYOS AND GEO. R. IRWIN

(Accepted for publication February 1, 1932)

The study of supplements for Bordeaux spray can hardly be called a neglected one. Holland, Dunbar, and Gilligan,[1] in Massachusetts, give an excellent review of the literature on this subject. They list 97 references and call attention to the fact that much of the evidence is contradictory. The present work is an attempt by laboratory studies to answer these questions: What effect does the use of a wetting agent have on the uniformity of the deposit on the leaf surface and what effect does it have on the total deposit per unit area?

The apparatus used in spraying the leaves consisted of a Hudson hand sprayer operated by 10-lbs. air pressure. The spray was directed against a perpendicular baffle board at a fixed distance from the tip of the nozzle. The spray was cut off close to the nozzle by a swinging shutter that was attached to a weighted pendulum in such a position that, when the shutter was in place to cut off the spray, the pendulum would be raised 45°. The plane of the arc described by the shutter-pendulum system was at right angles to the direction of the spray jet and parallel to the surface being sprayed. In operation, the spray jet would be directed against the perpendicular board and a point directly in the center of the area of deposition noted. The shutter would now be swung into position to cut off the spray and so held until the leaf to be sprayed was fastened to the baffle. A sheet of absorbent paper was used to keep the unsprayed side of the leaf clean. When the shutter was released, the pendulum-shutter system would swing through a long arc and, by adjusting the length of the pendulum arm, the time of each full period was made equal to 2 seconds. Thus, each swing of the pendulum represented 2 seconds of time that the spray was directed against the leaf. It was found that the shutter would return near enough to its original position to cut off the spray for 5 consecutive swings.

After spraying, the leaves were dried and sections cut from them with a cork borer 0.26 in. in diameter. These sections were analyzed for copper by the method described by Hockenyos in a previous publication.[2] In the tests where studies of uniformity of deposit were made, 9 sections were taken from each leaf according to a definite pattern and both the average deposit and the percentage irregularity calculated. The latter computa-

[1] Holland, E. B., C. O. Dunbar, and G. M. Gilligan. Supplements for copper fungicides. Massachusetts Agr. Exp. Sta. Bul. 252: 94–112. 1929.

[2] Hockenyos, George L. Solubility of Bordeaux. Phytopath. **21**: 231–234. 1931.

tion was made by finding the average of the deviation from the average deposit per section and dividing by the average deposit.

TABLE 1—*Showing how deposit of copper increases with time of spraying where 4–4–50 Bordeaux is used without a supplement. Uniform cherry leaves were used and sprayed on the upper surface at a distance of 13 in. from the nozzle*

Time in seconds	Copper per sq. in.	Percentage irregularity
12	.00011 gr.	10
16	.00020	18
20	.00015	38
24	.00010	16

In this test, it was noted that run-off began at 20 seconds, and that fact doubtless accounts for the drop-off in total deposit and the increase in irregularity at this point. It is interesting to note that according to Holland, Dunbar, and Gilligan,[3] Guba analyzed cucumber leaves for the amount of copper per sq. in. and found approximately 0.00009 gr. Considering the differences in method of spraying, nature of leaf surface, and methods of analysis, this may be regarded as good correspondence.

In a second test, apple leaves were sprayed and analyzed by the same method as in the above test, except that the leaves were only 11 in. from the tip of the spray nozzle. It was noted that run-off began in 6 seconds.

TABLE 2.—*Number of copper deposits and their analysis when blood is used as a wetting agent*

Time—seconds	Amount blood	Gr. copper per sq. in.	Heavy spots	Irregularity
	Per ct.			*Per ct.*
6	None	0.00009	None	29
6	"	0.00010	One (0.00066)	12
6	"	0.00010	Two (0.00038) (0.00016)	20
6	1/25	0.00011	None	10
6	1/25	0.00009	"	26
8	None	0.00010	"	34
8	"	0.00010	One (0.00050)	21
8	1/25	0.00010	None	14
8	1/25	0.00010	"	19

[3] *Loc. cit.*, page 105.

Bordeaux 4–4–50 alone was compared with Bordeaux 4–4–50 plus 1/25 per cent dried-blood albumin. The leaves were examined for heavy local deposits or spots, and, where such occurred, they were sectioned out separately and analyzed.

An examination of this table shows that the addition of blood albumin as a wetting agent did not decrease the amount of copper deposited but did eliminate the formation of heavy spots and to some extent increased the uniformity of the deposit.

From this table we see that the addition agents have had little effect on the amount of copper deposited and that the under surface of the leaf is capable of holding roughly twice as much copper as the upper surface,

TABLE 3.—*A comparison of the amount of copper deposited per sq. in. on peach leaves. Eight-second spraying time at 11 in. gave considerable run-off in all cases but did not insure uniform wetting. Bordeaux 4–4–50 was used in all cases*

Time—seconds	Supplement	Leaf surface	Gr. copper per sq. in.
	Per ct.		
8	None	Upper	0.000078
"	"	"	0.000081
"	"	"	0.000078
"	"	"	0.000090
"	"	"	0.000090
10	"	"	0.000111
"	"	Lower	0.000204
8	1 Colloidal clay	Upper	0.000075
"	"	"	0.000075
"	"	"	0.000087
"	"	"	0.000111
"	1/2 Soap	"	0.000090
"	"	"	0.000090
"	"	"	0.000084
"	"	"	0.000096
"	1/8 Kayso	"	0.000096
"	"	"	0.000090
"	"	"	0.000096
"	1/8 Gum ghetti	"	0.000066
"	"	"	0.000098
"	1/8 Waste pulp liquor	"	0.000090
"	"	"	0.000084
"	1/20 Blood albumin	"	0.000075
"	"	"	0.000090
"	"	"	0.000090
"	"	"	0.000093
"	"	"	0.000093
"	"	Lower	0.000216

whether or not a wetting agent is used. The wetting ability of each of the supplements used in table 3 was tested by noting with a stop-watch the number of seconds to give complete wetting of the entire leaf surface at a distance of 11 in. from the spray nozzle. The under surface of peach leaves was used and 6 leaves successively sprayed with each material.

TABLE 4.—*Showing wetting ability of the several supplements indicated in table 3, expressed in number of seconds required to completely wet entire leaf surface at a distance of 11 in. from spray nozzle*

Check—	Bordeaux alone	5,	45,	8,	8,	8, 10	seconds
Bordeaux plus	1/20 per cent	Blood albumin	6,	6,	6,	7, 6, 7	"
" "	1/8 " "	Kayso	8,	9,	5,	9, 5, 7	"
" "	1/8 " "	Gum ghetti	8,	12,	7,	14, 13, 25	"
" "	1/8 " "	Pulp liquor	8,	8,	15,	10, 7, 10	"
" "	1/2 " "	Soap	4,	4,	5,	5, 4, 5	"
" "	1 " "	Colloidal clay	15,	15,	15,	20, 13, 20	"

That a great variation exists in the resistance of leaves chosen for their apparent uniformity is readily seen. The soap, blood albumin, and Kayso are shown to greatly enhance wetting, but it will be noted in table 3 that the average deposit of copper is as heavy as when no supplement was used at all.

From the above data, it seems a reasonable conclusion that the addition of a wetting agent to Bordeaux will, to a certain extent, increase the uniformity of the deposit, will practically eliminate the deposition of heavy spots, and will not decrease the amount of copper deposited per unit area.

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FUNGICIDAL VALUE OF PINE-TAR OIL AND COPPER RESINATE

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(Accepted for publication March 2, 1932)

The use of petroleum oil as an insecticide has proven of great value to the entomologists in the control of orchard and garden insects. A serious criticism of this spray, however, has been its lack of fungicidal value, frequently necessitating two spray applications or attempts at tank combinations, such as Bordeaux and petroleum oil, which usually leads to a distinct lowering of the insecticidal value of the oil. This has been noted in the writer's experiments[2] and confirmed in a later paper.[3] The following typical examples of the need for combined oil-fungicidal sprays will serve to illustrate this:

Navel rot in oranges, especially prevalent in oil-sprayed citrus groves;[4] a dormant spray for peaches in the warmer districts where lime-sulphur injury frequently results from dormant application. The delayed dormant spray for apples where residual sulphur from lime-sulphur application leads to oil-sulphur injury in the early summer.

Research covering a period of 4 years has developed a pine-tar oil that, through factory treatment, is prepared specifically for use as a spray on plants. This special fraction has specifications rather similar to a number of other oils derived from the pine tree by destructive distillation and the use of steam, so, for convenience in designation, this fraction is known as "Palustrex," which name will be used throughout this paper. Palustrex is in itself a more active fungicide than the petroleum fractions used for foliage spraying, and, in addition to this property, it is an active solvent for copper resinate. Such combinations of oil and copper have the physical advantages of an oil spray that are not possessed by a water-carried application, the oil emulsion having a lower surface tension, being active as a wax solvent and capable of greater penetration than is possible with water-carried sprays, such as Bordeaux. It should be noted that this is a true solution of copper in oil and not simply a suspension, consequently

[1] Consulting Entomologist, San Francisco, California.

[2] Ong, E. R. de, Hugh Knight, and J. C. Chamberlin. A preliminary study of petroleum oil as an insecticide for citrus trees. Hilgardia **2**: 351–384. 1927. p. 362.

[3] Porter, B. A., and R. F. Sazama. Influence of Bordeaux mixture on the efficiency of lubricating-oil emulsions in the control of San Jose scale. Jour. Agr. Res. **40**: 755–766. 1930.

[4] Woglum, R. S., *et al.* Handbook of citrus insect control for 1930. California Fruit Growers' Exch., Los Angeles, Bul. 7: 1930. pp. 2–3.

the copper is carried in minute amounts wherever the oil penetrates, and for this reason lower concentrations of copper should be used than when applied as a suspension in water. Besides the fungicidal value, the antiseptic value of Palustrex as a preservative for oil emulsions made with organic emulsifiers has also been established as slightly superior to cresylic acid.[5]

Palustrex may be used alone in a water-soluble form both as a fungicide and as an insecticide for susceptible insects, such as aphids. For resistant forms, it becomes necessary to combine Palustrex with a suitable petroleum fraction to increase the viscosity and raise the evaporation point. Copper resinate and nicotine, if desired, may also be added. The wax solvency of such blends is improved by the addition of Palustrex, as shown by the following data: 98 per cent of a 0.3 gm. sample of apple wax dissolved in 3 cc. of Palustrex, the time required for complete solution being 2 minutes, the larger part dissolving almost instantly on stirring into the oil; 72 per cent of a similar 0.3 gm. sample of wax dissolved in 22 cc. of kerosene after 15 minutes' exposure; and 7 per cent of a similar wax sample dissolved in 19 cc. of lubricating oil after a period of 1 hour. In a test with beeswax it was found that a saturated solution of Palustrex contained 10 times as much wax as did kerosene.

Field tests of the copper-oil sprays have confirmed laboratory findings in that walnut blight has been checked to a greater extent with the dual-purpose sprays than was accomplished by standard Bordeaux. Similar results have also been noted in the mildew (*Erysiphe cichoracearum* DC.) attacking canteloupes, where the soluble Palustrex and copper proved more efficient than the standard Bordeaux spray. Extensive field tests are being made this winter with such oil-copper combination in the control of peach leaf curl and blight, brown rot, and other diseases.

The comparative toxicity of copper resinate and copper sulphate is indicated in the results shown in table 1. Such comparisons between copper resinate and copper sulphate cannot be made in an exact way since one is water-soluble and the other oil-soluble; consequently, the data does not represent the full value of the use of an oil-soluble material nor does it give opportunity for showing the results of superior physical values, including lower surface tension, wax solvency, etc. It may be noted, however, that for equal weights of the 2 copper salts the copper resinate gave almost equal value, although, as stated, in water suspensions the full physical values are not brought out nor does this take into consideration the variation in content of metallic copper.

Since copper resinate has not been in general use, the following brief discussion will be given regarding the comparative status of this salt and

[5] Ong, E. R. de. Present trend of oil sprays. Jour. Econ. Ent. 24: 978–985. 1931.

TABLE 1.—*Spore tests of the comparative toxicity of copper resinate and copper sulphate*

Toxic agent	Percentage of metallic copper	Spores	Spores germinated	Percentage germinated
Monilia				
Check 10 tests in distilled water	.....	260	192	73.90
20 tests with copper sulphate.....	25.4	557	20	3.59
20 tests with copper resinate.....	9.5	433	30	6.92
Botrytis				
Check 10 tests in distilled water	.....	235	147	62.50
20 tests with copper sulphate.....	25.4	529	25	4.73
20 tests with copper resinate.....	9.5	539	37	7.30

the more common copper sulphate. Copper sulphate has the following formula: $CuSO_4 \cdot 5H_2O$; it contains 25.4 per cent metallic copper and 36 per cent moisture. Copper resinate has the following formula: $Cu(C_{20}H_{29}O_2)_2$; contains 9.5 per cent metallic copper and no moisture. The lower content of metallic copper in the resinate salt is an advantage from the standpoint of metallic residues on both fruit and vegetables, which overcomes the criticism sometimes made in the past of too free use of Bordeaux. Copper resinate is very light and fluffy and somewhat sticky in nature from the high content of resin present. The physical nature of the salt makes it difficult to mix with oil, so the same companies that market the specially prepared pine-tar oil known as Palustrex are now putting out a paste containing 70 per cent copper resinate dissolved in 30 per cent Palustrex. This is comparable to lead ground in oil and obviates the mechanical difficulty of mixing and also the danger to the operator of working with a light copper dust that might be inhaled in excessive quantities.

Field tests of blends of Palustrex, petroleum, and copper resinate are being made as follows: 16 gal. of Palustrex, containing 25 per cent by weight of copper resinate, are added to 80 gal. of petroleum oil. This blend is then made up into an emulsion of 122 gallons. Using 5 gal. of this emulsion to 100 gal. of spray would give 0.158 per cent anhydrous copper resinate in the dilute spray. In a Bordeaux preparation containing 8 lb. of copper sulphate to 100 gal., there would be 0.65 per cent anhydrous copper sulphate in the dilute spray. The above formulas are for dormant spraying. These proportions are, of course, subject to further adjustment, particularly for specific purposes for which extensive work is planned on broad lines.

SUMMARY

A discussion of the present weakness of petroleum-oil sprays due to their lack of fungicidal value. The use of pine-tar oil, specially treated to promote safety on plants, as a general spray material and as a solvent for other toxic chemicals. The combination of the oil-soluble copper resinate with the treated pine-tar oil to give both insecticidal and fungicidal action.

SAN FRANCISCO, CAL.

PHYTOPATHOLOGICAL NOTES

Lolium Infected with Bunt of Wheat.[1]—Two species of Lolium were infected with bunt of wheat in artificial inoculation tests conducted in 1930–31. The seed of these species was coated with an inoculum that included both *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. The inoculum contained equal parts of the 10 physiologic forms previously described by the writer.[2] Three smutted heads of Lolium were found, 2 in *L. multiflorum* Lam., and 1 in *L. perenne* L. All 3 of these smutted heads contained spores that were typical of *T. levis.*

At the time the above tests were conducted the writer was quite unaware of the fact that as long ago as 1754 Tillet[3] conducted similar experiments in which he obtained positive results following the dusting of the seed of *Lolium* sp. with spores of the bunt of wheat. It is not known which species of Tilletia Tillet employed in his experiments. He also obtained positive results following the inoculation of clean wheat seed with the spores of bunt obtained from Lolium.

The writer[4] previously showed that it was possible to infect another genus, *Secale cereale,* with bunt from wheat. An attempt was made, therefore, to see if this organism would attack other grasses. In the fall of 1930 the following grass seeds were coated with the bunt inoculum described above: Cheat grass, *Bromus secalinus;* soft cheat, *B. hordeaceus* L.; smooth brome grass, *B. inermis* Leyss.; nodding wild rye, *Elymus canadensis* L.; English rye grass, *Lolium perenne;* Italian rye grass, *L. multiflorum;* timothy, *Phleum pratense* L.; meadow fescue, *Festuca elatior* L.; quack grass, *Agropyron repens* (L.) Beauv.; velvet grass, *Holcus lanatus* L.; proso millet, *Panicum miliaceum* L.; Kentucky blue grass, *Poa pratensis* L.; and hull-less gray oats, *Avena sativa* L. The cheat, Lolium, millet, and oats headed in 1931 but only the Loliums showed any infection. Removing the hulls before inoculation may enhance liability to infection.

The grasses were grown in the field in 4-ft. rows along with duplicate noninoculated check rows. No smut was found in any of the check rows. There were about 400 heads in each row of Lolium.

[1] Published as Technical Paper No. 179 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Farm Crops.

[2] Bressman, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oregon Agr. Exp. Sta. Bul. 281. 1931.

[3] Tillet, Mathieu. Dissertation sur la cause qui corrompt et noircit les grains de ble dans les épis et sur les moyens de prévenir ces accidents. 216 pp. Bordeaux. 1755.

[4] Bressman, E. N. Rye infected with bunt of wheat. Phytopath. **21**: 437–440. 1931.

Bunt may appear in some of the grasses that did not head in 1931. Unfortunately, much of this sod was destroyed, so it may not be possible to determine if any of the others produce smutted heads in 1932. It is hoped, however, that some additional results may be obtained in the inoculation trials.

A microscopic examination of the spores from the infected heads of Lolium showed that they were typical of *Tilletia levis* in respect to size, shape, and smoothness of exospore. The sori, when broken, produced an exceedingly strong odor typical of wheat bunt. Figure 1 shows part of a normal and bunted head of *L. multiflorum* and a normal and bunted seed (sorus).

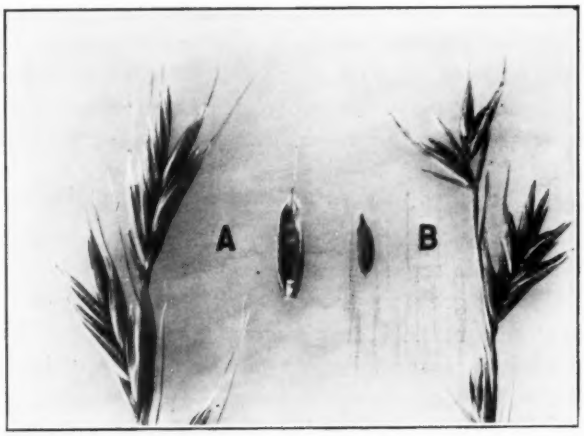

FIG. 1. Italian rye grass (*Lolium multiflorum*). A. Normal head and seed. B. Bunted head and seed.

Tilletia Lolii Auersw. has been described on Lolium. It may be a form of wheat bunt, but, if so, it is more closely related to *T. tritici*.

Large amounts of rye grass seed are produced in western Oregon each year. There have been no reports of Tilletia here, although a species of Ustilago or Melanopsichium has been noted. It appears that Tilletia is not common. It is not known whether the *T. levis* reported herein consists of more than one physiologic form. If only one form is present perhaps environmental conditions at time of seeding were optimum for its germination and growth. Additional inoculation tests with the *T. levis* obtained from Lolium, placing it back on hulled Lolium and on wheat, are being conducted—E. N. BRESSMAN, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Eriodendron as Host of Bacterium Malvacearum.—In a commercial planting of the well-known Mexican variety "Pochote" of *Eriodendron anfractuosum*—the kapok tree—on the Pacific coast of Guatemala, a rather serious bacterial disease caused by *Bacterium malvacearum* has been found. As this organism does not seem to have been reported from any host plant outside of the genus Gossypium, the purpose of the present note will be to put this occurrence on record, at the same time adding a short description of the disease symptoms, on the new host.

The disease is most prevalent during the rainy season and more destructive on young Pochote plants grown in seed beds; after transplanting into the open field the disease takes a less destructive course. Both leaves and stems may be affected by the disease.

On young infected leaves the symptoms are more conspicuous than on the stems. On the former, irregular spots surrounded by an oily translucent halo are formed a few days after infection has taken place. As the mesophyll is attacked it dries up and becomes dark brown. If the spots are located near the points of the digitate leaves, the points generally are killed, but the damage remains insignificant. In case the spots develop near the base of the leaflets, the infected leaflet will soon drop. Young plants of about 1 foot in height have been seen to become completely defoliated in a week. As the bud scales at the base of the young leaves may also become infected the infection is readily transmitted to the developing leaf, resulting in premature defoliation.

When full-grown leaves are infected, somewhat different symptoms develop. The infection seems to take place almost exclusively on the upper leaf surface. An oily zone, typical for lesions on young leaves, is not formed. The infection spreads in this case so slowly that a protective tissue can be generated in the mesophyll, which retards the extension of the lesion. The preventive layer often succeeds in keeping part of the mesophyll and the epidermis of the underside of the leaf free from the pathogen. In this connection it must be recalled that the fully developed Eriodendron leaf is thick and leathery. Leaf petioles are also subject to infection, which invariably leads to defoliation.

When stems of young Pochote plants—1–2 months old—become infected, oblong brown lesions develop rapidly. The center of such spots turns grayish when older, and the border zone often appears slightly raised over the surface because of extensive formation of wound cork. Often such lesions remain superficial and accordingly cause only slight damage to the stem. If the growth of the stem is rapid, as it is during prolonged wet weather, lesions on the stem near the growing point may extend into the xylem. A girdle of necrotic tissue forms around the main axis, thereby causing the death of the growing point. This leads in its turn to the formation of one or several new shoots below the girdle. As this procedure may be repeated

several times during the growing season, the whole habitus of the Eriodendron tree may become completely changed. A healthy young tree normally attains a height of about 5 feet before the first whirl of branches is formed. In case of repeated girdling of successive bud shoots, a stunted, irregularly branched dwarf tree results. Such trees are, of course, useless for planting purposes.

Similar bacterial lesions have also been found near the growing point on the horizontal branches of older trees. The result is the same as just described, with the difference, however, that the new shoots, instead of growing horizontally, like the mother branch, grow upwards at a right angle to the supporting branch. This negative geotropism of adventitious Eriodendron shoots is always displayed when horizontal branches have been seriously wounded and have given rise to new shoots.

A series of cross-inoculation experiments proved that *Bacillus malvacearum* from cotton readily infected the Eriodendron, causing lesions of the type described above in 6 to 10 days. Infections with the bacterium from Eriodendron similarly caused infection on Gossypium.—B. T. PALM, *Department of Botany, University of Illinois, Urbana, Illinois.*

A Note on Entyloma Dahliae Syd. from Sumatra and Guatemala.—A disease of the cultivated Dahlia, caused by *Entyloma Dahliae* Syd., has recently been causing some alarm to horticulturists on the Continent of Europe. Originally described from South Africa, the Entyloma has subsequently, some years ago, been found also in Spain and in Holland. At present the fungus would seem to be spreading quite rapidly over the European Continent, having been reported of late at least from Belgium, France, and Germany by pathologists in the respective countries.

To the above list of countries can now be added Sumatra (Dutch East Indies) where the Dahlias, which are cultivated there only at higher altitudes, were found rather badly affected by this Entyloma disease in a number of mountain localities; these observations were made in 1924 and 1925. In all cases the plant material had been introduced from Holland by several importers, indicating that the disease must be fairly wide-spread in the last-mentioned country.

As *Entyloma Dahliae* seems to belong to the growing number of pathogens that, upon introduction, show a rapid extension over the Continent of Europe, the following tentative suggestion regarding its country of origin may be of interest.

The Dahlias are, as is well known, natives of Mexico and Guatemala, where they occur at altitudes of 6,000 ft. and higher above sea-level. During a stay in Guatemala the writer had the opportunity of studying two species, *Dahlia excelsa* Benth. and *D. coccinea* Cav., both of which are quite common on the volcanoes near Antigua. It was found that a species of

Entyloma, which to all purposes agreed very well with the descriptions of *E. Dahliae* Syd., occurred, often in great abundance, on *D. coccinea*, not, however, on *D. excelsa*. The same fungus was found prevalent also on cultivated Dahlias in gardens around the city of Guatemala. After the first indication of this limited host range had been obtained, during several growing seasons (1927–29) a search was made for the Entyloma on *D. excelsa*, always, however, with the same negative result. This fact probably is not without significance, as *D. coccinea* is regarded as one of the ancestors of our cultivated Dahlia, whereas *D. excelsa* would seem to have been introduced into cultivation but recently in Mediterranean countries. Recent importations of the former species to botanical gardens or by horticultural establishments for crossing purposes may thus possibly account for the introduction and subsequent distribution of *Entyloma Dahliae* on cultivated forms of Dahlia.—B. T. PALM, *Department of Botany, University of Illinois, Urbana, Illinois.*

An Undescribed Loose Smut of Barley. As a result of further studies on the infection of barley by the loose-smut fungus through seed inoculation discovered by Tisdale and Tapke,[1] it has been found that loose smut of barley is caused by either of two fungi: (1) *Ustilago nuda* (Jens.) Kell. and Sw. and (2) a darker-spored species for which the name **Ustilago nigra** n. sp. is proposed. The two smuts are readily separable, as shown below.

Bases of comparison	*Ustilago nuda*	*Ustilago nigra*
Color of spore mass	Olivaceous brown	Dark chocolate brown
Individual spores		
Color	Golden brown	Dusky brown
Size	5.5 × 6 μ	6.5 × 7 μ
Duration of viability[a]	3 to 6 months, rarely 1 year	Over 18 months
Control of loose smut in plants from seed from inoculated flowers following treatment of the seed with Ceresan dust or liquid formaldehyde (1–320, seed soaked 90 minutes)	No control	Complete control
Ability of the fungus to cause seedling infection resulting from inoculation of mature seed with chlamydospores	No seedling infection	Seedling infection

[a] Limited studies.

[1] Tisdale, W. H., and V. F. Tapke. Infection of barley by *Ustilago nuda* through seed inoculation. Jour. Agr. Res. **29**: 263–284. 1924.

In the light of the above, the divergent results obtained by investigators in the production of loose smut of barley through dusting mature seed with chlamydospores and in the control of loose smut through seed treatment with surface disinfectants, might be explained.—V. F. TAPKE, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Plant Pathology at the Fourth Congress of the International Society of Sugar Cane Technologists in Puerto Rico.—The 4th Congress of the International Society of Sugar Cane Technologists was held in Puerto Rico, Mar. 2 to 16, 1932. The section on diseases of sugar cane was attended by G. Wilbrink, of Java; A. F. Bell, of Australia; Atherton Lee, of Philippine Islands; J. P. Martin, of Hawaii; E. W. Brandes and E. V. Abbott, of the United States, and Melville T. Cook, C. E. Chardon, R. A. Toro, Arturo Roque, and J. G. Harrer, of Puerto Rico.

Three sessions were devoted entirely to diseases of sugar cane and a joint session was held with the entomologists.

Thirty papers were presented, including an international survey of the diseases of sugar cane, among which were 7 papers on virous diseases; 7 on bacterial diseases; 2 on root diseases; and 12 papers on other subjects. A 5-day excursion through the sugar-cane districts of the Island gave an excellent opportunity for the study of the diseases in the field and to visit the 2 experiment stations. The papers will be published in the proceedings of the society. The next Congress will be held in Australia in 1935.—MEL. T. COOK, *Insular Experiment Station, Rio Piedras, Puerto Rico.*

Citrus Mildew.—In PHYTOPATHOLOGY **9**: 266, a correction is made on citrus mildew by Mr. T. Petch to a note over his name in PHYTOPATHOLOGY **5**: 350–352, in which he indicates that this fungus, which closely agrees with *Oidium tingitaninum* Carter, by which name it is now usually referred to in Ceylon literature, does not occur in Ceylon on pummelo (*Citrus maxima* Merr. = *C. decumana* L.).

A recent revision of the various Citrus species on which this mildew has been observed makes a further correction desirable. Citrus mildew is now commonly found in Ceylon on pummelo (*Citrus maxima* Merr.), grapefruit (*C. maxima* Merr. var. *uvacarpa* Merr. & Lee), as well as on sweet orange (*C. sinensis* Osbeck), lemon (*C. limonum* Risso), and Mandarin orange (Citrus species undetermined) as previously recorded. In addition, it has been found also on the Kalamondin orange (*C. mitis* Blanco) and, more commonly, on the Ceylon sour orange (Citrus species undetermined). No perithecial stage of this Oidium has been observed up to date. It has been found that this mildew can be very satisfactorily controlled by regular weekly sprayings during damp warm weather with certain lime-sulphur mixtures.—W. C. LESTER-SMITH, Department of Agriculture, Peradeniya, Ceylon.

BOOK REVIEW

WAKSMAN, SELMAN A. *Principles of Soil Microbiology.* XXVIII + 894 pp., XV pls., 83 figs., 2nd Ed. Thoroughly Revised. Williams and Wilkins Company, Baltimore, Md. 1932. Price $10.00.

Research workers and teachers in soil microbiology are well acquainted with the first edition of this book. For the information of others, it may be said that Waksman brought together the available information on soil microbiology, the first time that such a review of literature on the subject has appeared in English. The works of the Russian and other foreign investigators whose publications are generally inaccessible to the average worker were cited and their results summarized in the discussion of the various subjects.

In the preface to the second edition, the author states, "Within the brief period of four years, since the appearance of the first edition of this book, the numerous contributions to our knowledge of the rapidly growing subject of microorganisms and their activities in the soil necessitate a number of changes in the new edition. . . . . The author availed himself of the criticisms which have been so freely given in the various reviews of the first edition . . . .".

It is only natural that the second edition of a work of this kind would have many changes. New material is constantly becoming available, which, of course, means another edition in a few years. The typographical errors and the mistakes in citations in the first edition have been largely corrected, although some persist, such as spelling Shantz as Shanz throughout the book. In such a large undertaking, minor mistakes perhaps should be overlooked (author index—E. J. Russell XV should be E. J. Russell XIV; F. Löhnis XVI should be F. Löhnis XV, may serve as illustrations).

The author has adhered to the original plan of the work, dividing it into four major parts.

Part A. The soil population. Occurrence and abundance of microorganisms in the soil. Two chapters.

Part B. Isolation, identification, and cultivation of soil microorganisms. Twelve chapters.

Part C. Chemical activities of microorganisms. Seven chapters.

Part D. Soil microbiological processes and soil fertility. Thirteen chapters.

In comparison with the first edition, Part A has one more chapter; Part B, one less; Part C, two less; and Part D, four more.

In order not to increase the size of the book but still include the literature of the past four years, it was necessary to omit something. Waksman

chose to omit the titles of the original papers, to combine certain chapters, and to omit others. Several new chapters, however, have been added, so that actually the saving of space is due mainly to the omission of the titles of the references cited. A great many will undoubtedly criticize the author for doing this instead of finding some other way of limiting the size of the volume. However, to many this will be of minor importance, since the work is indexed according to author and subject.

The criticism might be offered that a number of citations are often placed under one reference number at the bottom of the page without any distinguishing marks except that the volume numbers are in black-face type. From the view-point of the reader, this is unfortunate, especially, since some of these citations may be referred to on subsequent pages. The summaries at the end of certain chapters appear to add nothing of value and might be omitted.

The new edition contains about a thousand more references than the first and a few more figures and tables. There are four less plates. Plate I has new illustrations and plates V and VI have been changed and improved.

The task of collecting and summarizing the results of researches on soil microbiology can be appreciated only by those who attempt to keep abreast of the science. Therefore, the value of the book under discussion need not be proclaimed; it speaks for itself.—NATHAN R. SMITH, Soil Microbiology, U. S. Department of Agriculture, Washington, D. C.

ABSTRACTS OF PAPERS DUE NOVEMBER 15

November 15 has been designated as the closing date for receipt of manuscript for phytopathological abstracts. This means that abstracts must reach the Secretary not later than November 15. They must not exceed 200 words in length and three typewritten copies are necessary. Indicate time required for presentation and whether stereopticon will be needed.

In editing these the committee on abstracts will apply the following rules:

1. Not more than two abstracts under sole or senior authorship of one person will be accepted.
2. Previously published material must not be included.
3. Abstracts must not exceed 200 words.